Интеграция клеточного метаболизма: общие механизмы

Integration of metabolism

1. **Energetic integration** (NTPs (mainly ATP) are the universal currency of energy; main electron carriers in oxidative reactions are NADH and $FADH_2$, main electron donor in reductive biosynthesis is NADPH)

Integration of metabolism

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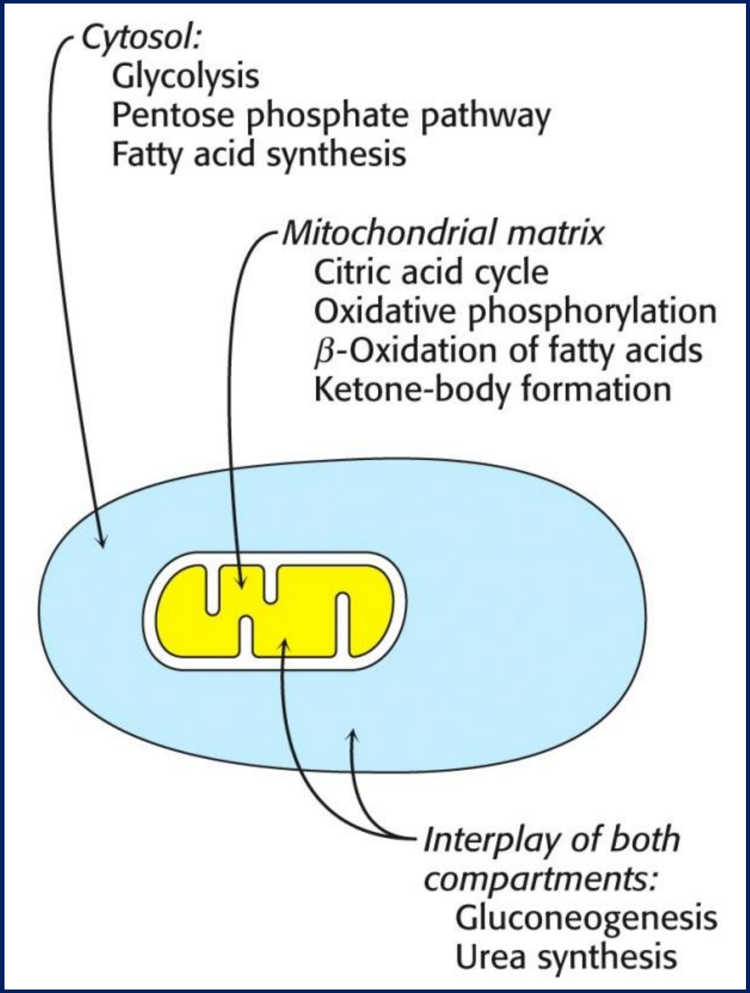
2. **Metabolic reactions integration in pathways** (few key metabolites, that are key junctions/nods of metabolic pathways; biomolecules are constructed from a small set of building blocks; enzymes' activity is tightly regulated)

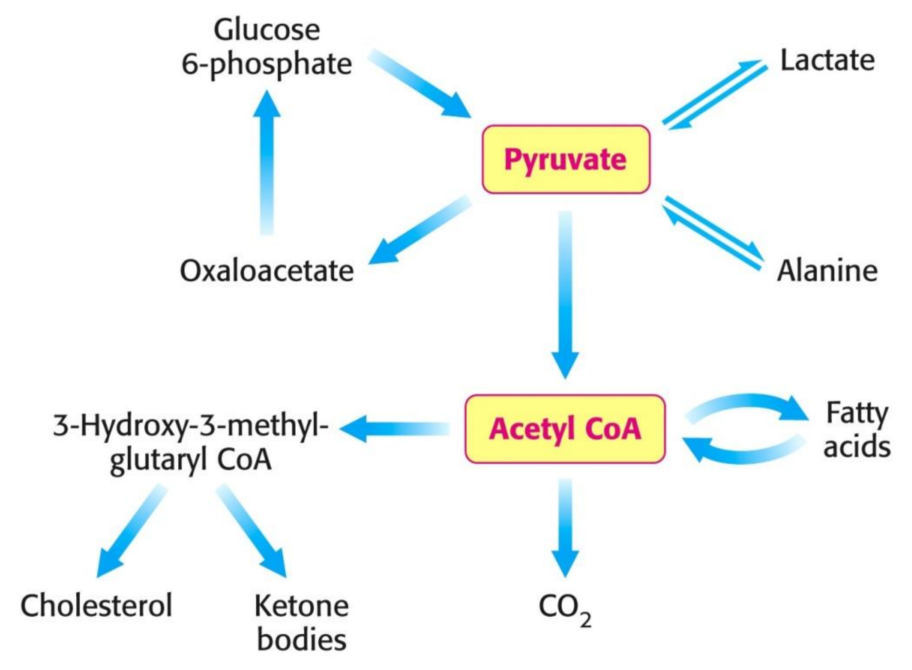
Key Junctions between Pathways



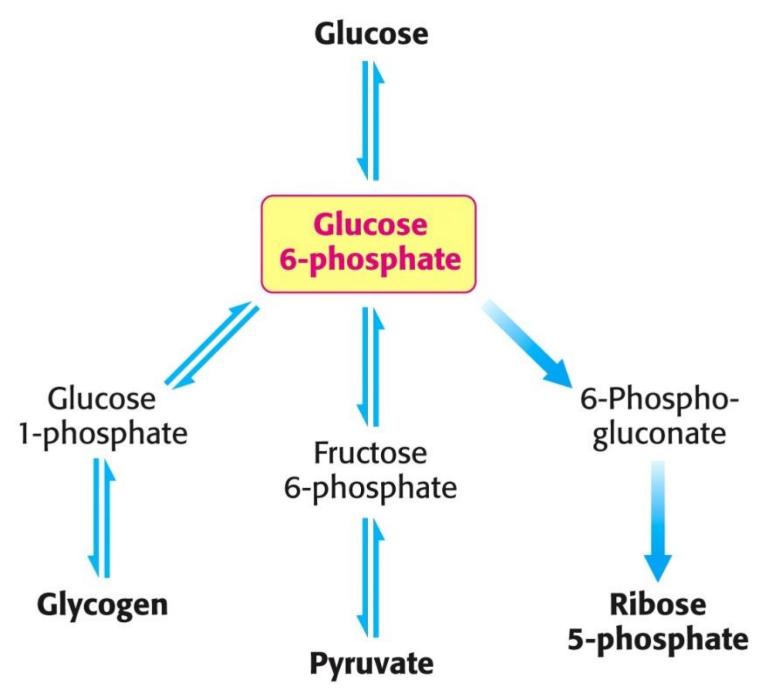
Cytosol:
Glycolysis
Pentose phosphate pathway
Fatty acid synthesis

Mitochondrial matrix
Citric acid cycle
Oxidative phosphorylation
β-Oxidation of fatty acids
Ketone-body formation

Interplay of both compartments:
Gluconeogenesis
Urea synthesis

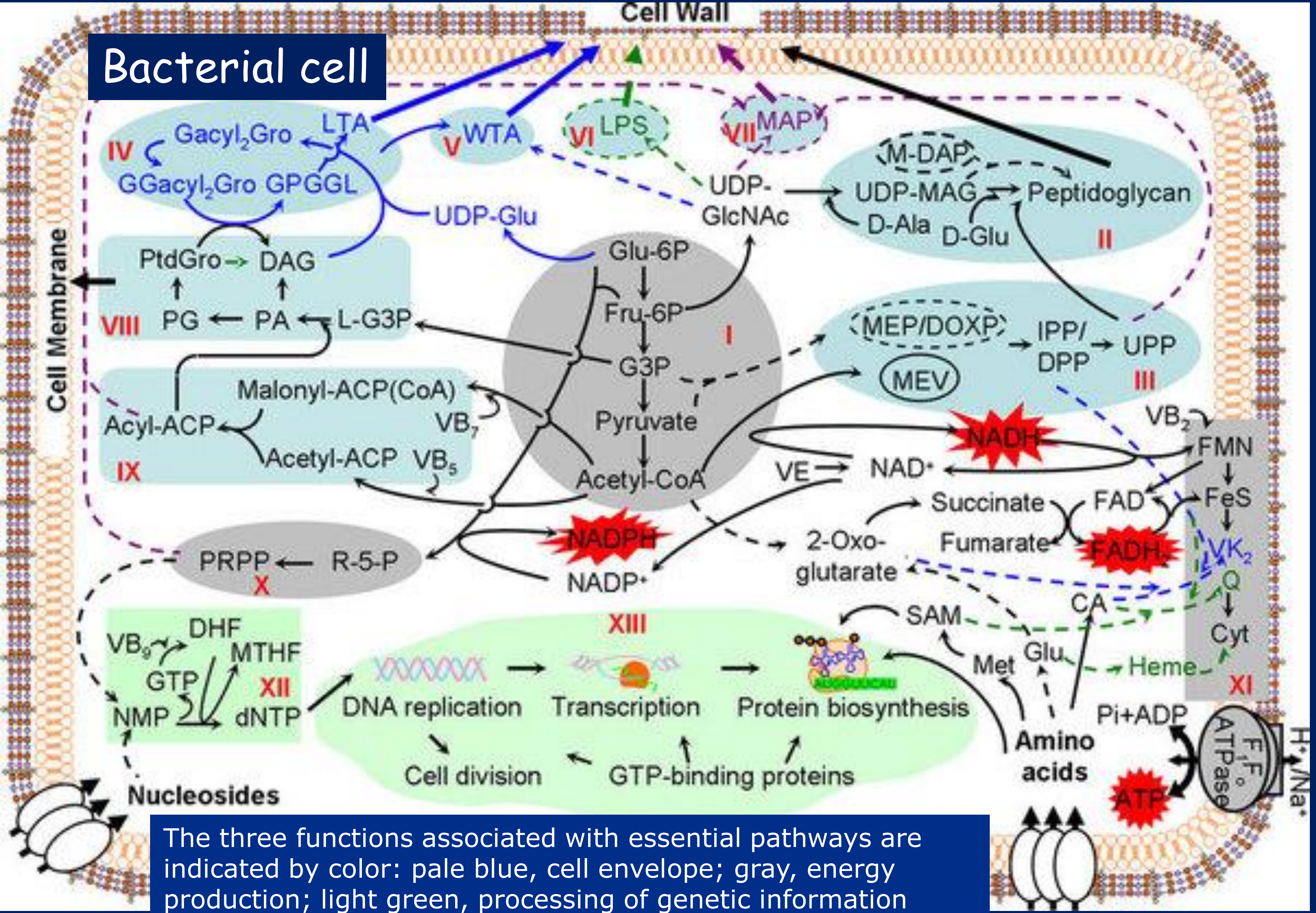


Glucose 6-phosphate
Lactate
Pyruvate
Oxaloacetate
Alanine
3-Hydroxy-3-methyl-glutaryl CoA
Acetyl CoA
Fatty acids
Cholesterol
Ketone bodies
CO_2



Glucose
Glucose 6-phosphate
Glucose 1-phosphate
Fructose 6-phosphate
6-Phosphogluconate
Glycogen
Pyruvate
Ribose 5-phosphate

Bacterial cell



The three functions associated with essential pathways are indicated by color: pale blue, cell envelope; gray, energy production; light green, processing of genetic information

Integration of metabolism

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3. **Regulation of metabolism through enzymatic activity**

Regulation of enzymatic reactions

- Regulation of enzyme *structure* as protein
- Regulation of enzyme *quantity* by change of gene expression or rate of degradation
- Regulation of *pH and physical conditions* for reaction
- Action of *inhibitors or activators*
- Existence of *isoenzymes*
- Regulation *of substrates* and/or *cofactors/coenzymes* concentration and access by ***compartmentation and redistribution*** (transport, competing between enzymes, binding)

Integration of metabolism

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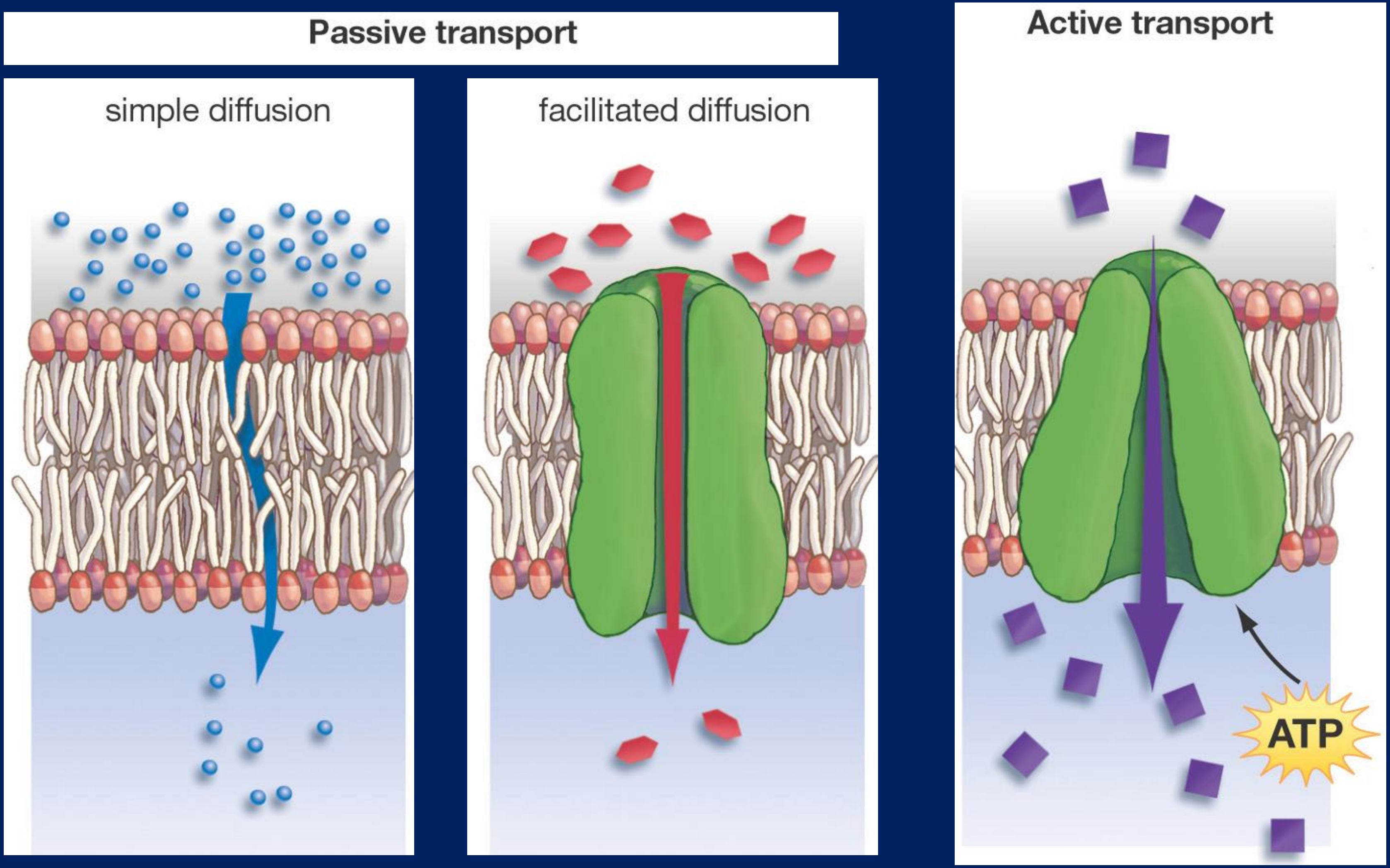
3. Regulation of metabolism through enzymatic activity
4. **Regulation of transport/flow of substrates**

Three Forms of Transport Across the Membrane



Example: Oxygen or water diffusing into a cell and carbon dioxide diffusing out.

Examples: Glucose or amino acids moving from blood into a cell.

Examples: Pumping Na$^+$ (sodium ions) out and K$^+$ (potassium ions) in against strong concentration gradients.

Gated Channels

- Control Transmembrane Potential
- Voltage Gated
- Ligand Gated

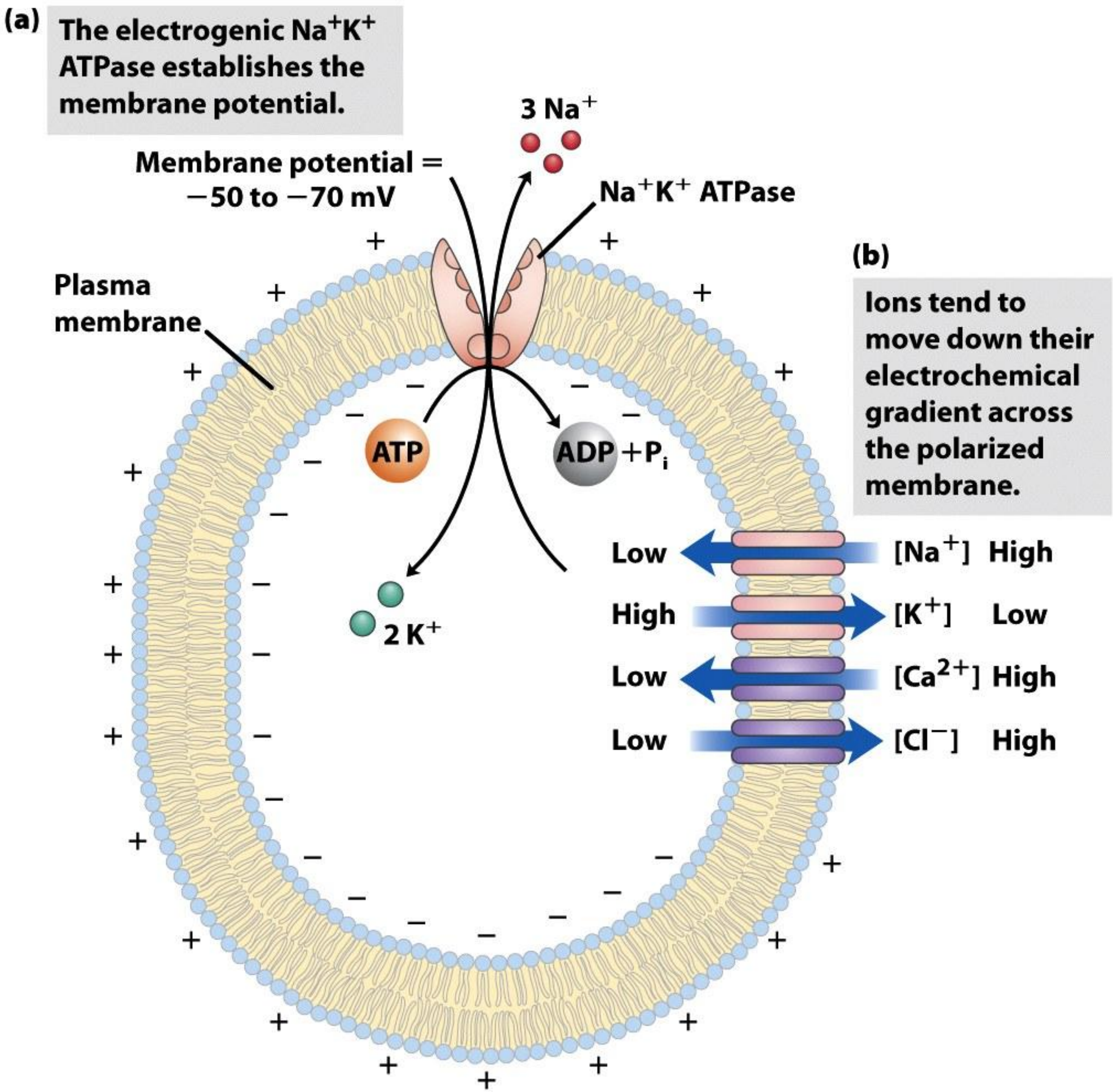

(a)
The electrogenic Na⁺K⁺ ATPase establishes the membrane potential.
3 Na⁺
Membrane potential = −50 to −70 mV
Na⁺K⁺ ATPase
Plasma membrane
ATP
ADP + Pᵢ
2 K⁺
(b)
Ions tend to move down their electrochemical gradient across the polarized membrane.
Low [Na⁺] High
High [K⁺] Low
Low [Ca²⁺] High
Low [Cl⁻] High

Figure 12-24
Lehninger Principles of Biochemistry, Fifth Edition
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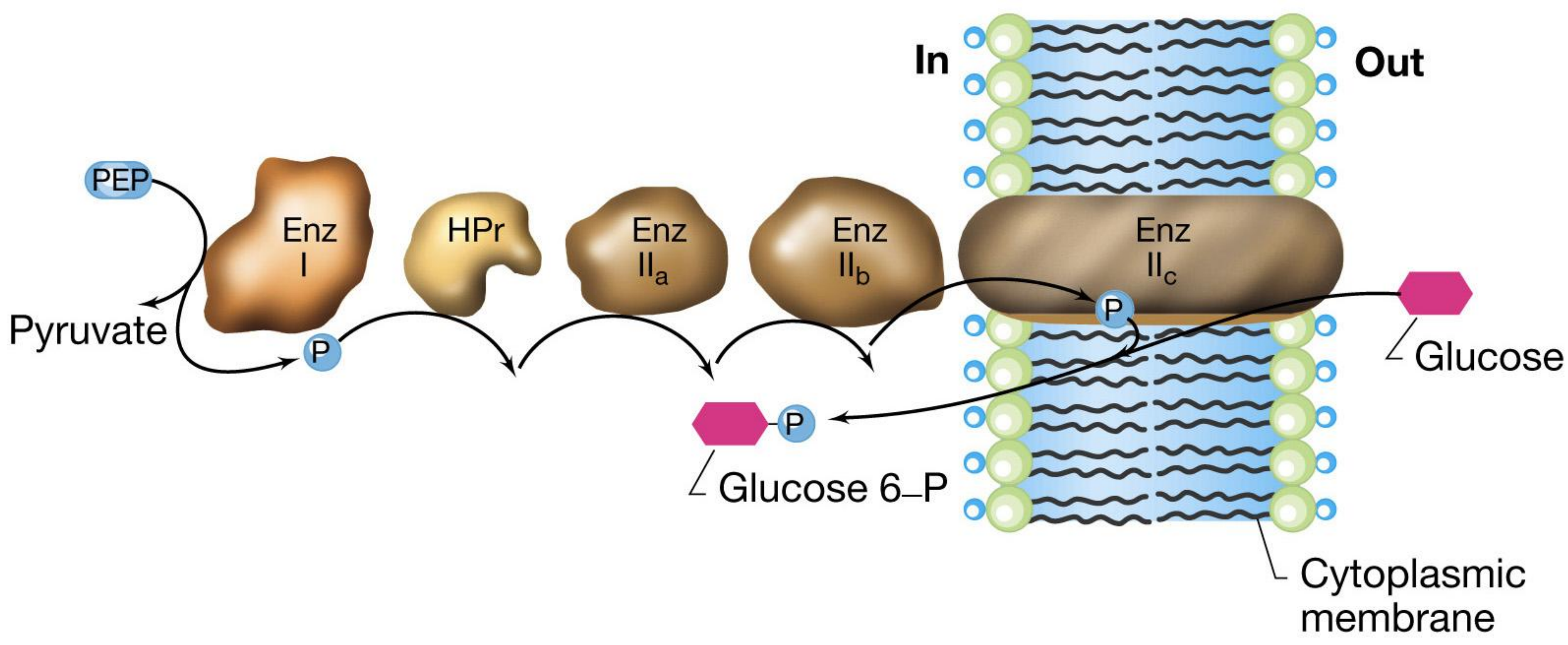
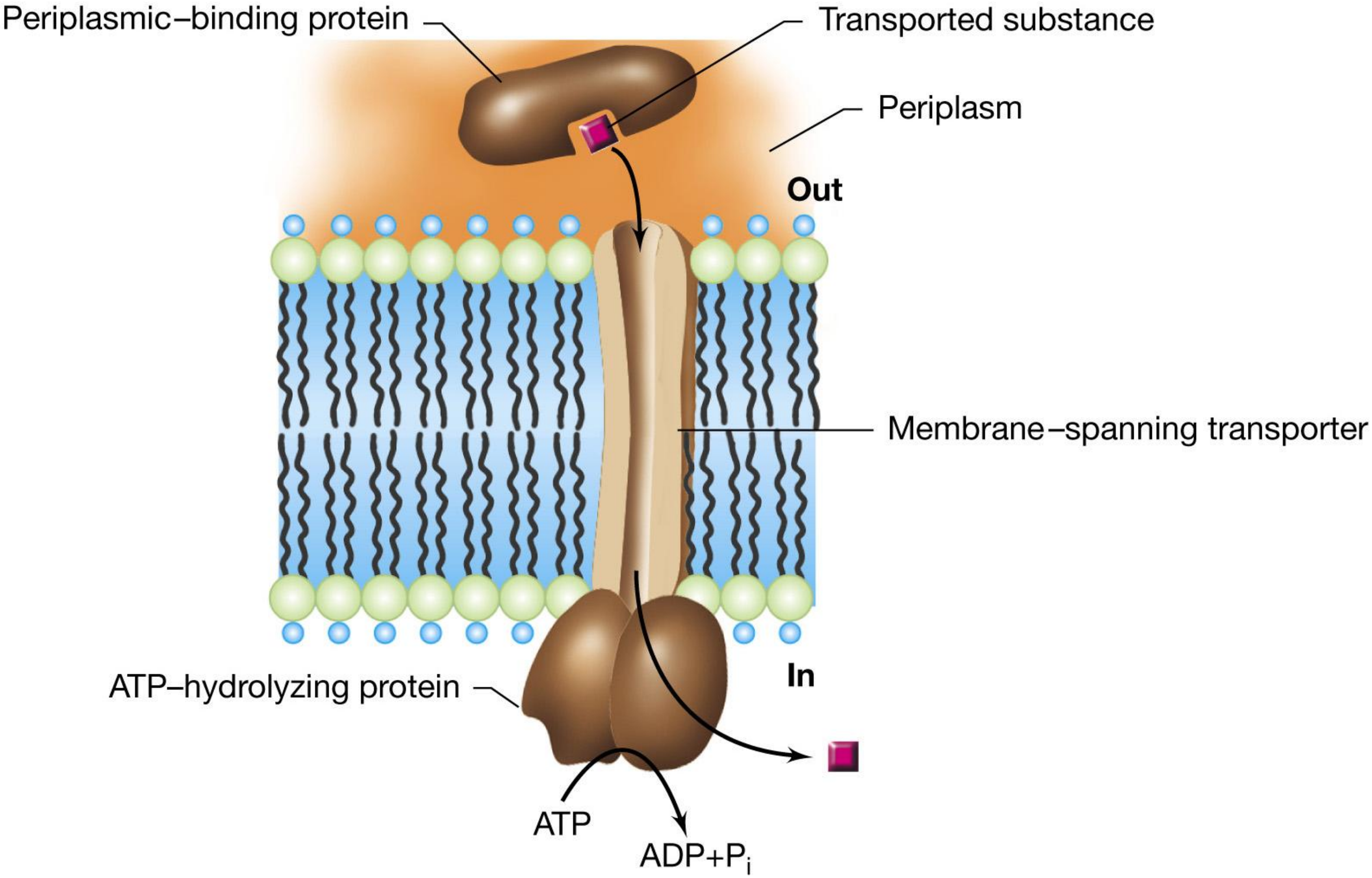
Group translocation



ATP-Binding Cassette (ABC-type) transporter



Integration of metabolism

1. **Energetic integration** (NTPs (mainly ATP) are the universal currency of energy; main electron carriers in oxidative reactions are NADH and $FADH_2$, main electron donor in reductive biosynthesis is NADPH)
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3. Regulation of metabolism through enzymatic activity
4. Regulation of transport/flow of substrates

→ 3 and 4 Based first of all on protein activity regulation (→regulation of metabolites flow and enzymatic reactions velocity)

Regulation of protein 3D structure

Is based on change of 3D structure.

a. **Non-enzymatic effects**

- Physical factors (T°C, hν, osmotic pressure, membrane potential, etc) → sensors.
- Molecules-regulators (ligands: nucleotides, metals, sugars, AA, peptides, etc; oxidants; gases) → Allosteric regulation. Reversible
- Association-dissociation of other protein (subunits). Reversible.

b. **Enzymatic modifications**

- Covalent modification (phosphorylation, adenylation, acetylation, methylation, etc). Usually reversible. Need pair of enzymes – e.g. kinase - phosphatase; acetylase - deacetylase. These enzymes usually are regulated *by ligands*
- Partial proteolysis (removal of peptide). Irreversible. Usually – activation.

Protein modifications:
formation or breakage of covalent bonds

- disulfide bond formation
- addition of a small moiety
  - phosphorylation, glycosylation, methylation, hydroxylation, *etc*.

usually specified by amino acids at certain positions, or various degenerate or specific motifs found in proteins

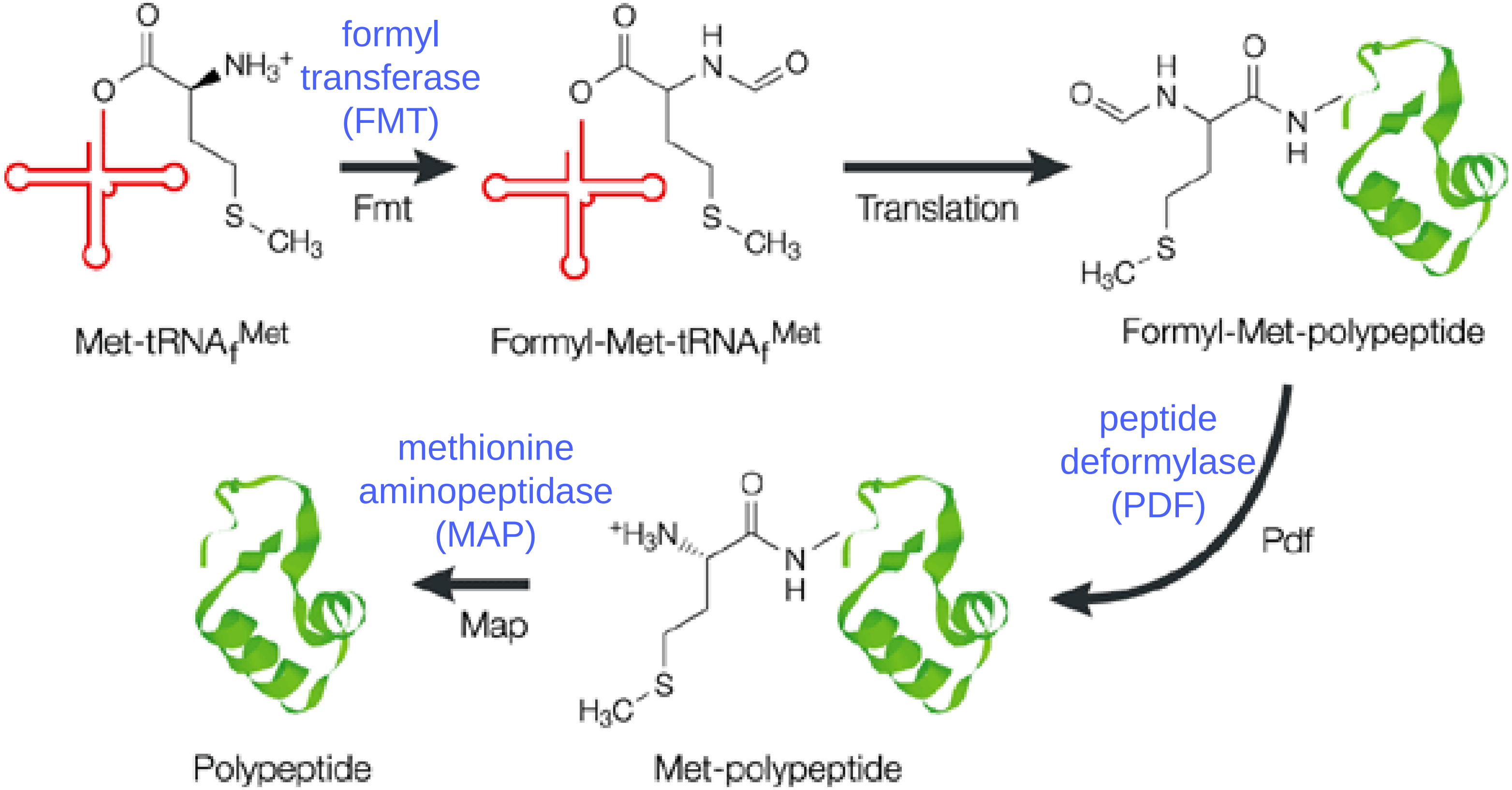
- truncation
  - self-cleavage

    intramolecular chaperone
  - cleavage of signal sequences - signal peptidases
  - protein splicing: inteins
  - intermolecular cleavage (*e.g.*, by proteasome)
- addition of ubiquitin or ubiquitin-like protein

N- and C-terminal modifications: acylation

- ***frequently, the N-terminal methionine is not present in mature proteins***

- **Acylation** includes acetylation, formylation, pyroglutamylation, myristoylation
  - ~80% eukaryotic cytosolic proteins are acetylated at their N-termini
  - makes N-terminal (Edman) sequencing difficult without special treatment
    - requires enzymatic removal or treatment with chemicals that may cleave labile peptide bonds
  - formyl group occurs mostly as modification of the initiator methionine in bacteria
  - pyroglutamate represents a cyclic amide generated from an N-terminal glutamic acid or glutamine residue
    - can be generated by spontaneous cyclization but could also be an artifact of protein isolation under slightly acidic conditions
  - myristoylation is a co-translational lipid modification that is common to many signalling proteins; occurs only on N-terminal glycine residues


NH3+
O
O
S
CH3
Met-tRNAfMet
formyl transferase (FMT)
Fmt
H
N
O
Formyl-Met-tRNAfMet
Translation
Formyl-Met-polypeptide
H3C
peptide deformylase (PDF)
Pdf
+H3N
Met-polypeptide
methionine aminopeptidase (MAP)
Map
Polypeptide

N- and C-terminal modifications: methylation and amidation

- Methylation of N-terminal amino groups is rare; different methylases do modify specific proteins, including ribosomal proteins
    - methylation of ribosomes affects their function

- Amidation of peptides (*e.g.,* hormones) sometimes occurs at the C-terminus Amidation is the addition of an amide group to the end of the polypeptide chain

Modification of individual side chains: phosphorylation and glycosylation

- Phosphorylation
  - phosphorylation can affect the activity and structure of proteins
  - perhaps as many as 1 in 8 proteins are phosphorylated
  - too many examples to list: e.g. HSF activity is modulated by phosphorylation; cell-signalling molecules are best characterized

- Glycosylation
  - glycosylation takes place in the ER, golgi by a variety of enzymes
  - glycosylated proteins often found on the surface of cells or are secreted
  - folding/assembly of glycosylated proteins requires ER molecular chaperones

  - addition of GlcNAc (beta-O-linked N-acetylglucosamine) residues occurs in the cytoplasm and nucleus

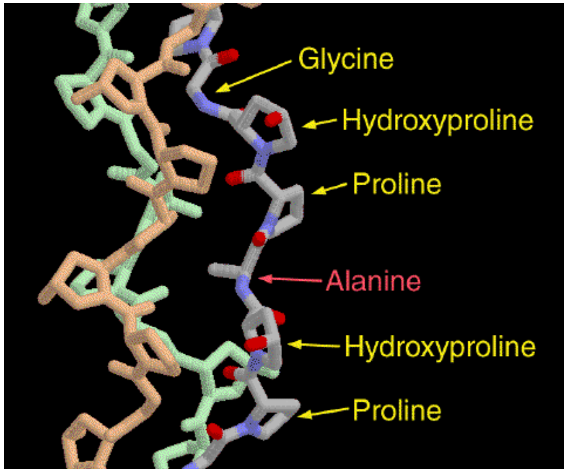
    - modifications are carried out by O-linked GlcNac transferases (OGTs)

    - proteins modified by O-GlcNAc include:

    cytoskeletal proteins, hormone receptors, kinases & other signalling molecules, nuclear pore proteins, oncogenes, transcription factors, tumor suppresors, transcriptional & translational machinery, viral proteins

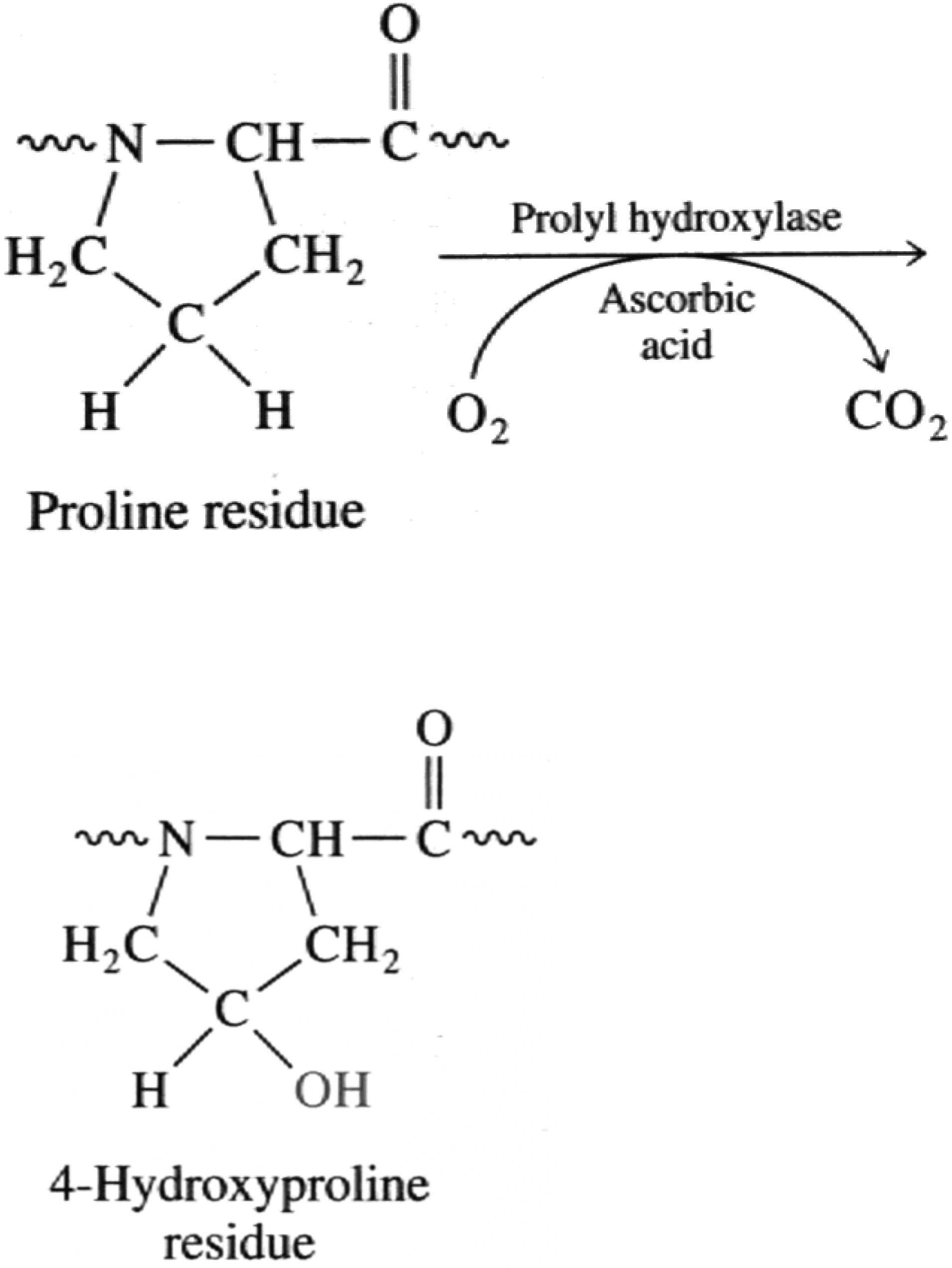
Modification of individual side chains: various others

- Prenylation, fatty acid acylation
  - proteins without major hydrophobic (transmembrane) domains can be directed to membranes by prenylation of their C-terminal cysteine residue



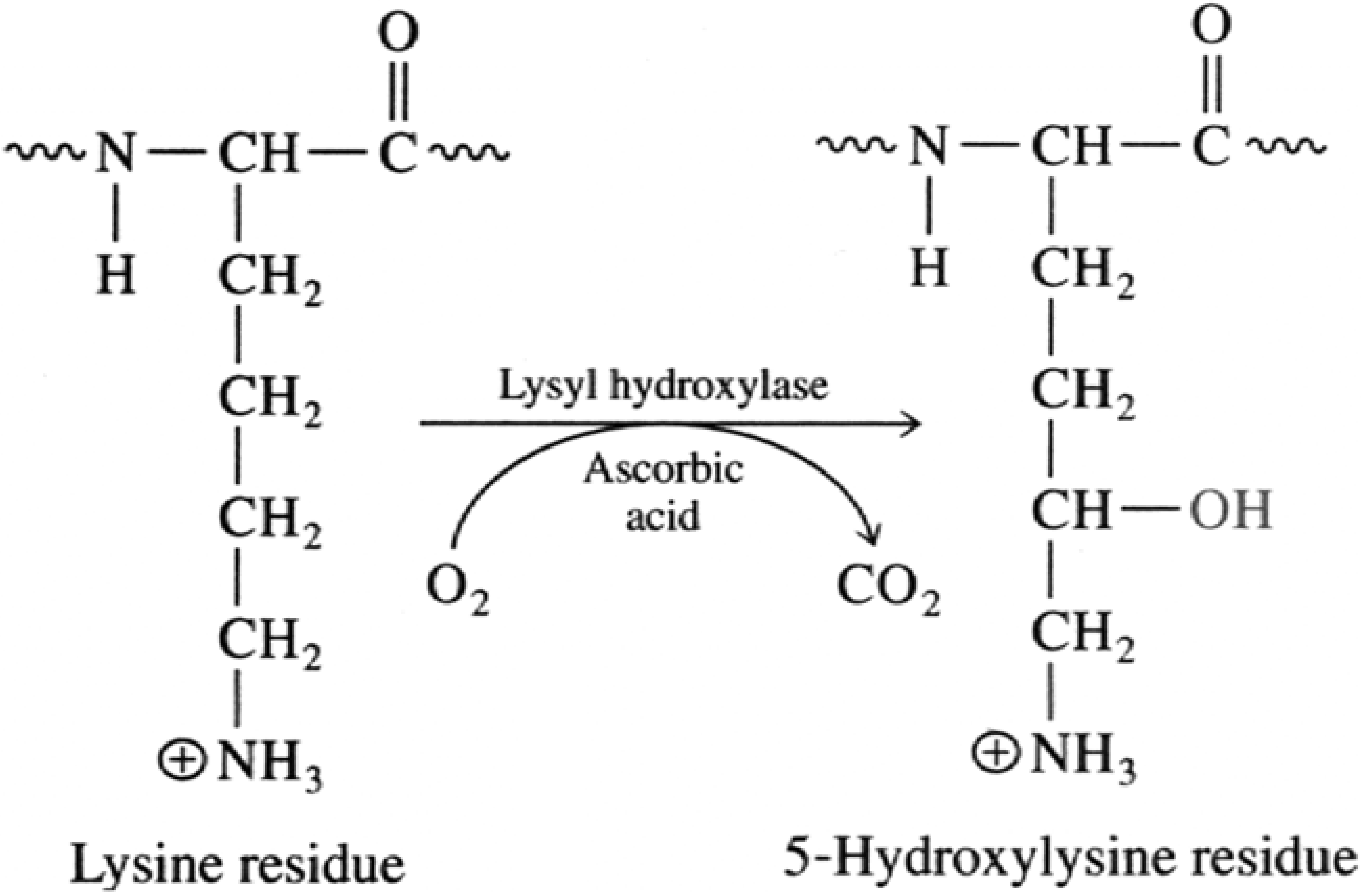
- Hydroxylation and oxidation, carboxylation
  - a variety of derivatives are known; *e.g.*, hydroxyamino acids (hydroxyproline) are very common in collagen

- Selenocysteine/selenomethionine modification
  - essentially all selenium in cells occurs as selenocysteine
  - selenomethionine is a useful too for protein crystallography: can grow cells in the presence of the modified amino acid and produce protein containing se-Met; can deduce ‘phase’ of protein this way



The presence of hyp residues greatly increases the potential for H-bonding between chains.

Hyp and pro make up 25% of the residues of collagen

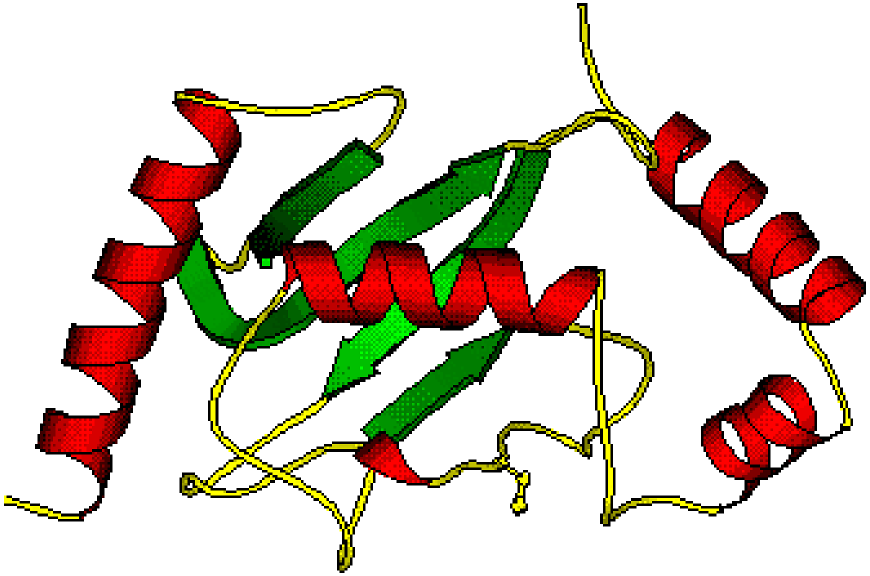


Some Hydroxy lysine residues are covalently bonded to carbohydrates making collagen a glycoprotein

SUMO

Small Ubiquitin Related Modifier (SUMO)

- SUMO does not have the Lys-48 found in ubiquitin
- SUMO does not make multi-chain forms
- SUMO-1,2,3 are the mammalian forms
- SUMO-1, 101 amino acids, C-terminal Gly, 18% identical to ubiquitin.

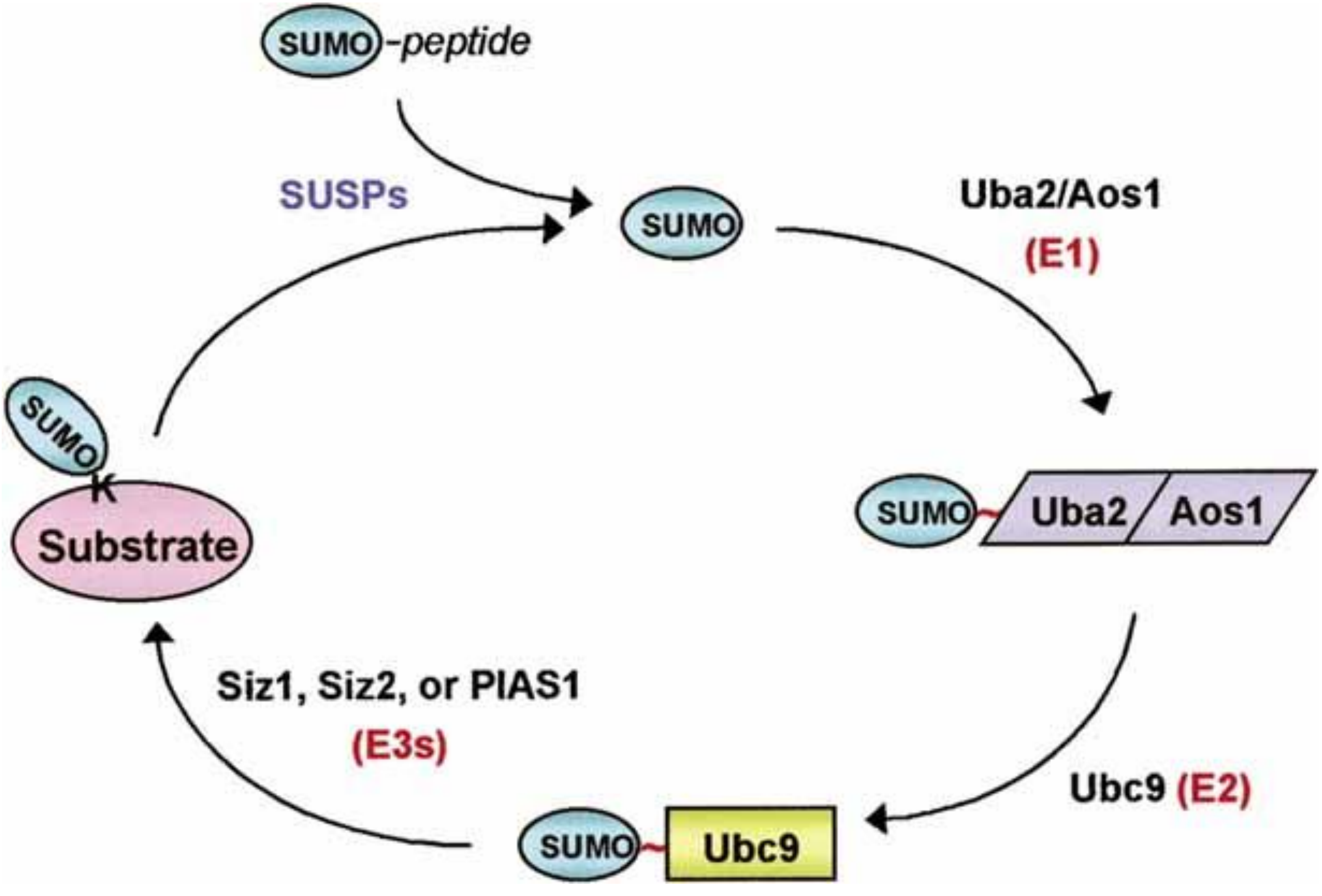


SUMO Enzymes

1. SUMO-activating enzyme: heterodimer
2. SUMO-conjugating enzyme: Ubc9, an E_2 enzyme that can’t be ubiquitinated
3. SUMO-ligase: E3 enzyme that is specific for sumoylation

E3-like proteins increase affinity between SUMO-Ubc9 and target proten

Sumoylation and Desumoylation Cycle with E1-,E2-,E3-like Enzymes



Biological Roles of SUMO Modification

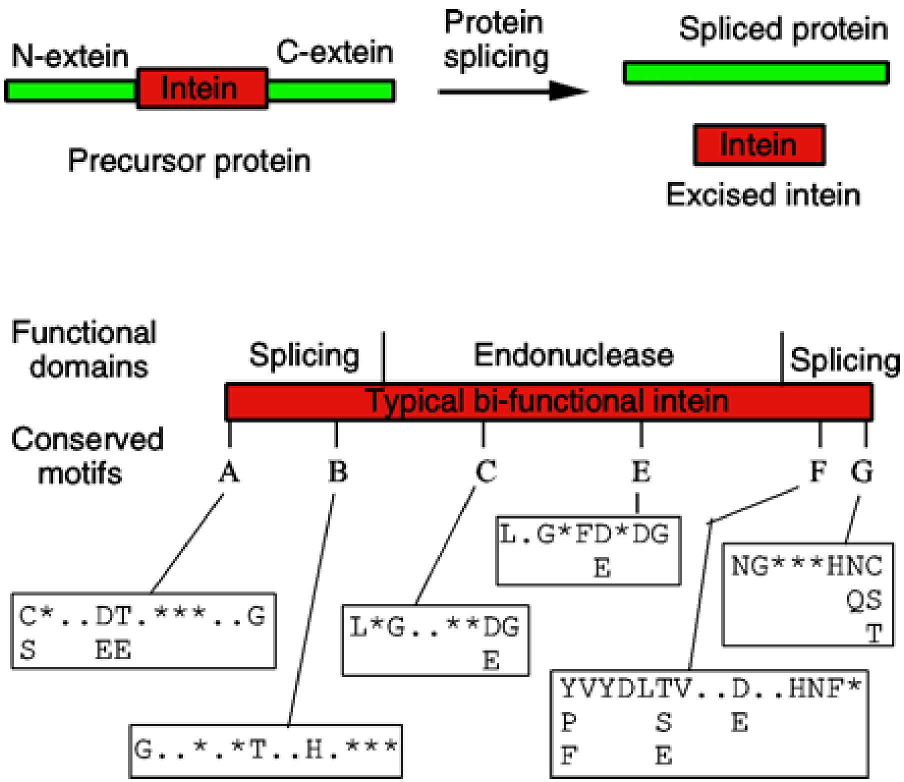
- Antagonistic role against ubiquitin: IκBα is sumoylated preventing ubiquitination and degradation (NF-κB pathway).
- Protein translocation: Ran-GAP1/RanBp2
- Modulation of transcriptional activity: activates transcriptional activity of p53.
- Subnuclear structure formation: protein targeting to nuclear bodies. PML, tumor suppressor.

Proteins Modified BY SUMO

Protein modified by SUMO	Function
RanGAP1	Nuclear transport
PML	Affected by translocations causing leukemias
IκBα	Inflammatory response
p53	Tumor suppressor
Mdm2	Regulates p53
Werner’s syndrome protein	Mutations causing premature aging
Septins	Polarized cell growth and cytokinesis

Protein splicing: inteins

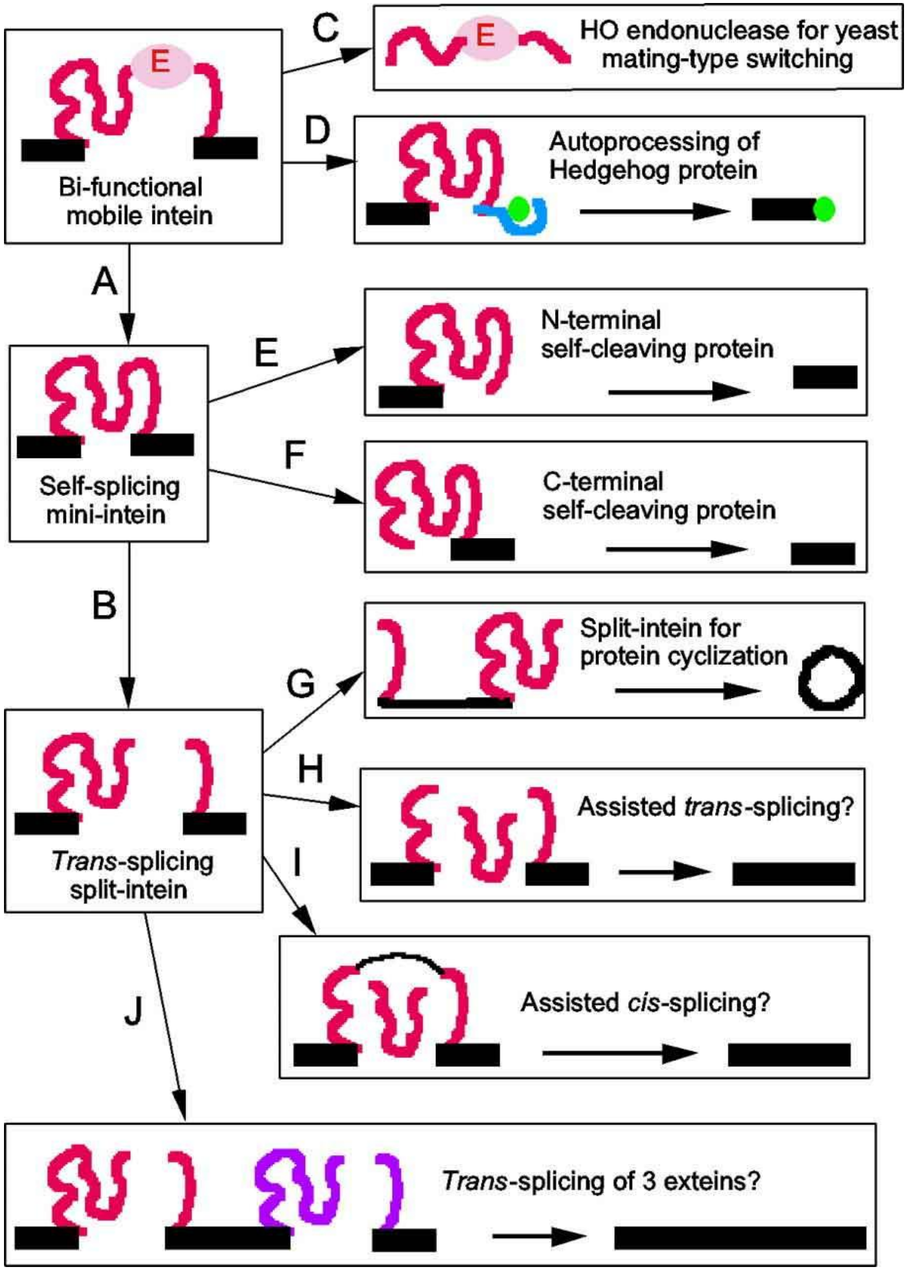
- Inteins represent the **protein** equivalent to the genomic **DNA** intron: elements that are spliced out of the final (mature) product
- unlike introns, inteins are self-splicing through the use of an endonuclease
- used commercially in biochemical applications (*explanation on board*)



- inteins are 134-608 aa
- the mini-inteins do not have the endonuclease domain but have other characteristics of inteins

Legend:
Schematic illustration of protein splicing (*upper part*) and intein structure (*lower part*). The two terminal regions of the intein sequence form the splicing domain of a typical bifunctional intein. Six conserved sequence motifs (*A* to *G*) are shown. The intein sequence begins with the first amino acid of motif A and ends with the second last amino acid of motif *G*. Motifs *C* and *E* are the dodecapeptide motifs of endonuclease. A star (*) stands for hydrophobic amino acids (V, L, I, M). A dot (.) stands for a nonconserved position.
adapted from Liu, X.-Q. (2000) *Annu. Rev. Gen*

Protein splicing: inteins


E
Bi-functional mobile intein
C
E
HO endonuclease for yeast mating-type switching
D
Autoprocessing of Hedgehog protein
A
Self-splicing mini-intein
E
N-terminal self-cleaving protein
F
C-terminal self-cleaving protein
B
Trans-splicing split-intein
G
Split-intein for protein cyclization
H
Assisted trans-splicing?
I
Assisted cis-splicing?
J
Trans-splicing of 3 exteins?

Legend:
Scenarios of intein evolution. *A*, loss of the endonuclease domain. *B*, breaking the intein sequence. *C*, loss of the splicing function. *D*, replacing the C-extein with a cholesterol molecule (*green dot*). *E*, loss of C-terminal cleavage and splicing. *F*, loss of N-terminal cleavage and splicing. *G*, placing intein fragments on two ends of a protein. *H*, breaking intein into 3 fragments. *I*, separating a middle fragment of the intein from the rest. *J*, presence of two different split inteins.

Scenarios *A* to *D* are based on examples observed in nature.

Scenarios *E* to *G* are based on engineered artificial inteins.

Scenarios *H* to *J* are purely hypothetical.

Results of change of 3D structure

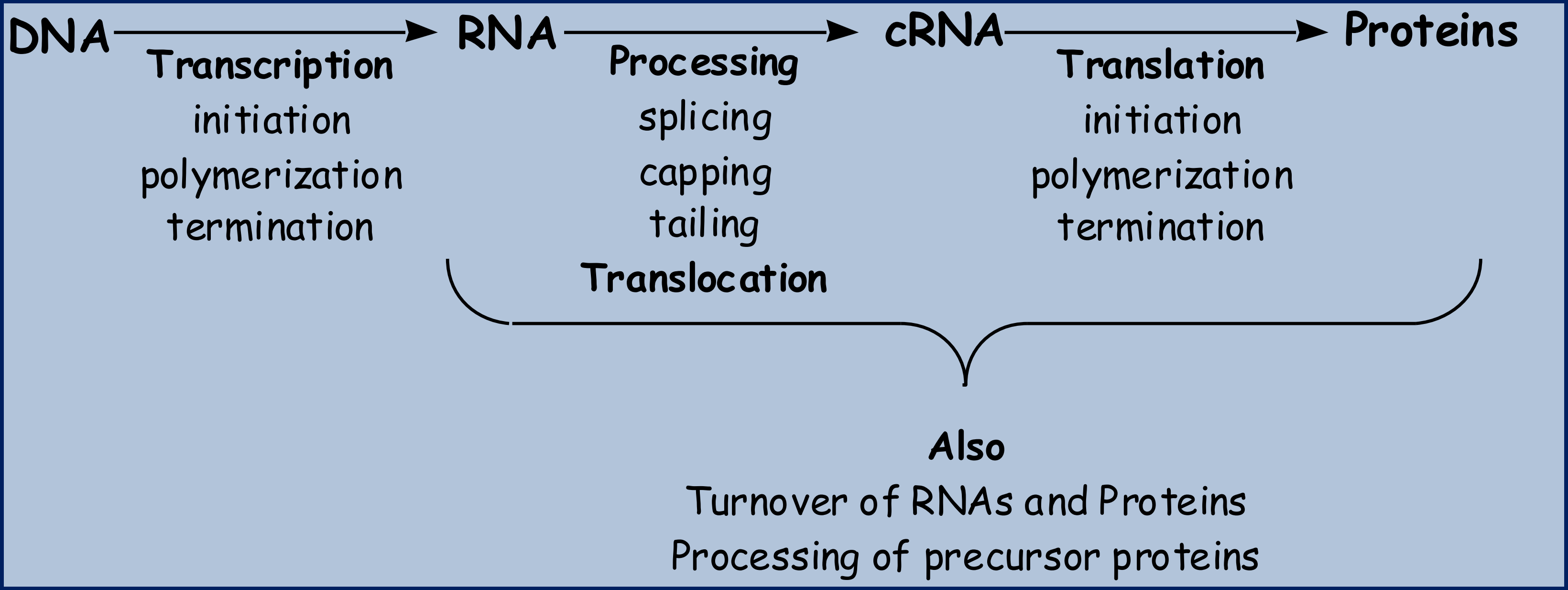
→ +/- of affinity

- to ligands (signal transduction; transport),
- to proteins (assembly-disassembly),
- to NA (gene expression)

→ closing-opening of channels (transport, potential)

→ +/- of catalytic activity

Sites of Regulation of protein synthesis



DNA → **RNA** → **cRNA** → **Proteins**

Transcription
initiation
polymerization
termination

Processing
splicing
capping
tailing
Translocation

Translation
initiation
polymerization
termination

Also
Turnover of RNAs and Proteins
Processing of precursor proteins

Thanks to *in silico* analysis and genome sequencing

How to generate diversity?

Non coding regions increased with complexity

Genomes	Size	genes (Nb)	ORF (%)
Bacteria			
E. Coli	4,6	4289	88
Archea			
P. abyssi	1,76	2065	91
Eucarya			
S. cerevisiae	13	6400	68
A. thaliana	125	25500	29
C. Elegans	100	18000	27
D. melanogaster	180	13600	13
H. Sapiens	3000	24500	1,4

→ sRNA
1-2%

→ Intron
encoded RNAs
miRNA…

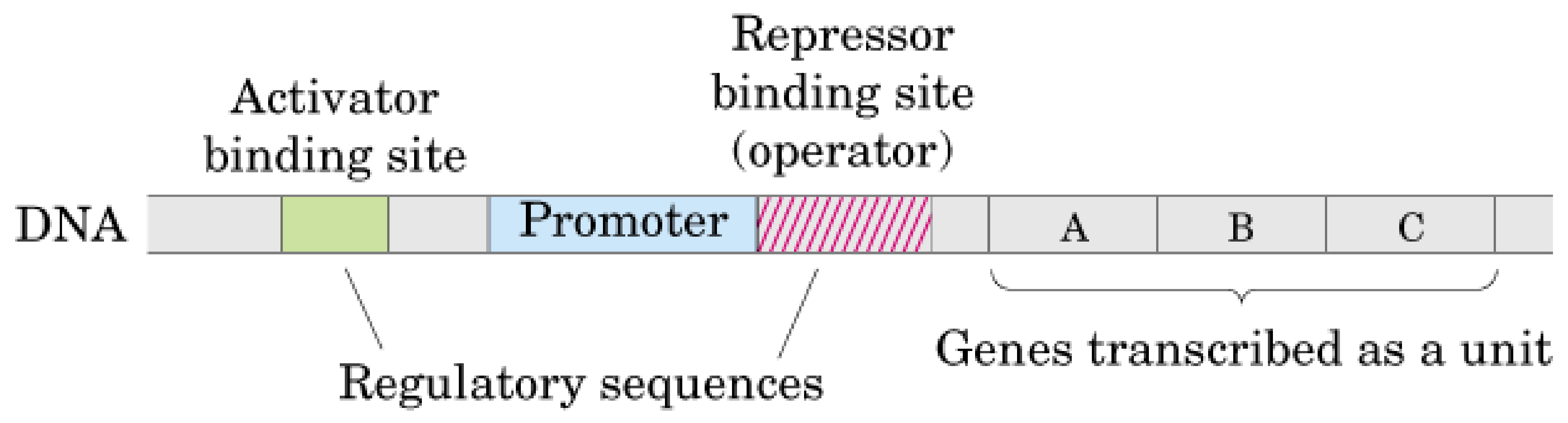
Gene Regulatory Mechanisms

- Transcriptional Mechanisms
  - Type of promoters & RNA polymerase (pro- + eukaryotes)
  - Control of Transcription (Constitutive / Inducible/ Repressible)
  - Transcription Factors and TFBS
  - Transcription attenuation → riboswitches (only prokaryotes)
- Epigenetic Mechanisms (only eukaryotes)
  - DNA methylation at CpG sites
  - Histone acetylation
  - Chromatin remodeling
- RNA processing (only eukaryotes)
  - Capping
  - Splicing and Alternative Splicing
  - Poly-Adenylation
  - RNA degradation rates
  - RNA export to cytoplasm
- Translation inhibition and mRNA degradation (only eukaryotes)
  - MicroRNAs and small interfering RNAs (siRNAs or RNAi) degrading mRNA

Gene Regulatory Mechanisms

- **Transcriptional Mechanisms**
  - **Type of promoters & RNA polymerase (pro- + eukaryotes)**
  - **Control of Transcription (Constitutive / Inducible/ Repressible)**
  - **Transcription Factors and TFBS**
  - **Transcription attenuation → riboswitches (only prokaryotes)**

Representative prokaryotic operon


Repressor binding site (operator)
Activator binding site
DNA
Promoter
A
B
C
Regulatory sequences
Genes transcribed as a unit

Regulons, modulons and stimulons

Regulon

More than one operon is under the primary control of the same regulatory protein (ex. the maltose operon)

Modulon

Group of genes that can respond to a common regulatory protein even though they may also be members of different regulons

Stimulons

Group of genes hat all respond to the same environmental signal. Large group of genes and very complex regulatory pathways.

Global Control Networks

- Cells can control several regulons by employing *alternative sigma factors*.

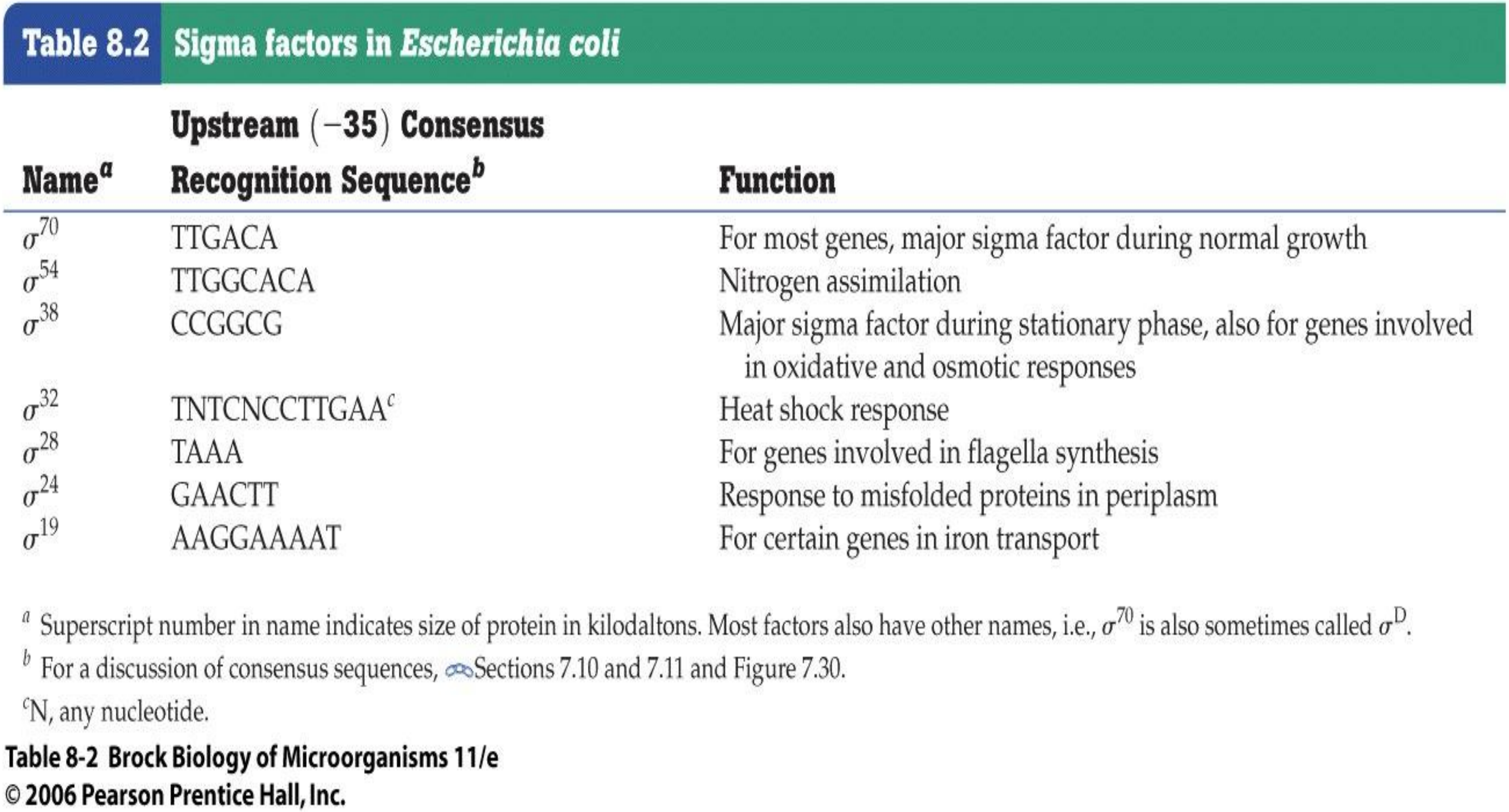


Table 8.2 Sigma factors in *Escherichia coli*

Name[a]	Upstream (−35) Consensus Recognition Sequence[b]	Function
σ^{70}	TTGACA	For most genes, major sigma factor during normal growth
σ^{54}	TTGGCACA	Nitrogen assimilation
σ^{38}	CCGGCG	Major sigma factor during stationary phase, also for genes involved in oxidative and osmotic responses
σ^{32}	TNTCNCCTTGAA[c]	Heat shock response
σ^{28}	TAAA	For genes involved in flagella synthesis
σ^{24}	GAACTT	Response to misfolded proteins in periplasm
σ^{19}	AAGGAAAAT	For certain genes in iron transport

[a] Superscript number in name indicates size of protein in kilodaltons. Most factors also have other names, i.e., σ^{70} is also sometimes called σ^{D}.

[b] For a discussion of consensus sequences, Sections 7.10 and 7.11 and Figure 7.30.

[c] N, any nucleotide.

Table 8-2 Brock Biology of Microorganisms 11/e

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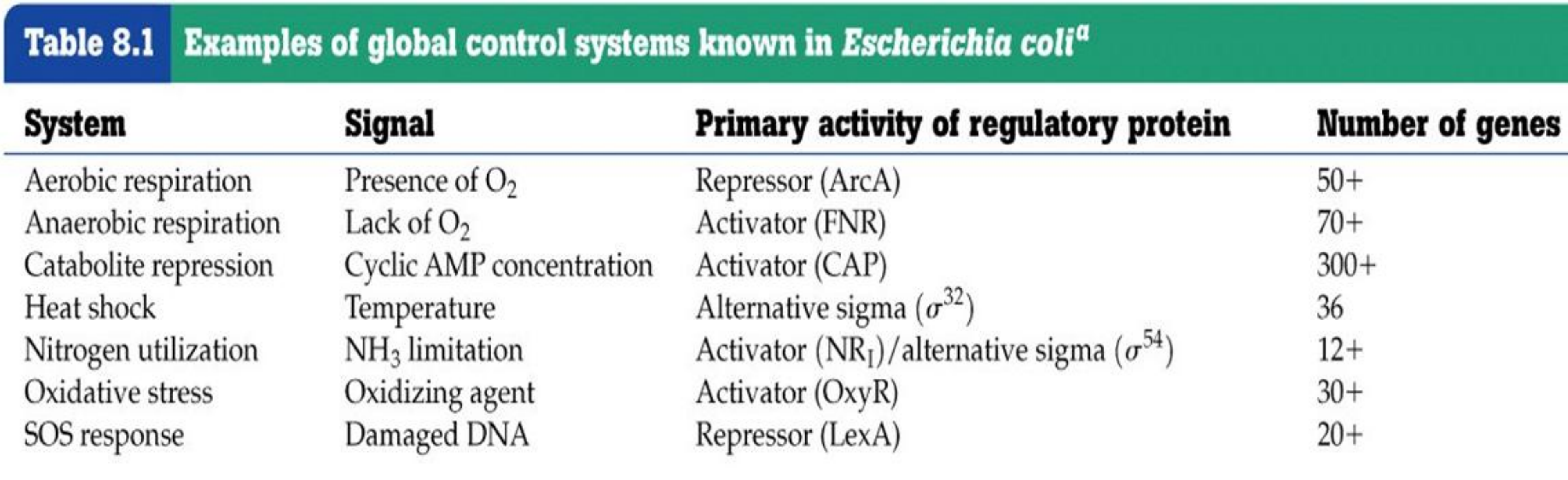


Table 8.1 **Examples of global control systems known in *Escherichia coli*[a]**

System	Signal	Primary activity of regulatory protein	Number of genes
Aerobic respiration	Presence of O_2	Repressor (ArcA)	50+
Anaerobic respiration	Lack of O_2	Activator (FNR)	70+
Catabolite repression	Cyclic AMP concentration	Activator (CAP)	300+
Heat shock	Temperature	Alternative sigma (σ^{32})	36
Nitrogen utilization	NH_3 limitation	Activator (NR_I)/alternative sigma (σ^{54})	12+
Oxidative stress	Oxidizing agent	Activator (OxyR)	30+
SOS response	Damaged DNA	Repressor (LexA)	20+

Different operons/regulons affected by same environmental signal

- Change from O_2 to anaerobic growth
- Presence of glucose
- Oxidative stress
- Nitrogen limitation; phosphate starvation
- Stationary phase; entering starvation state

Heat Shock Response

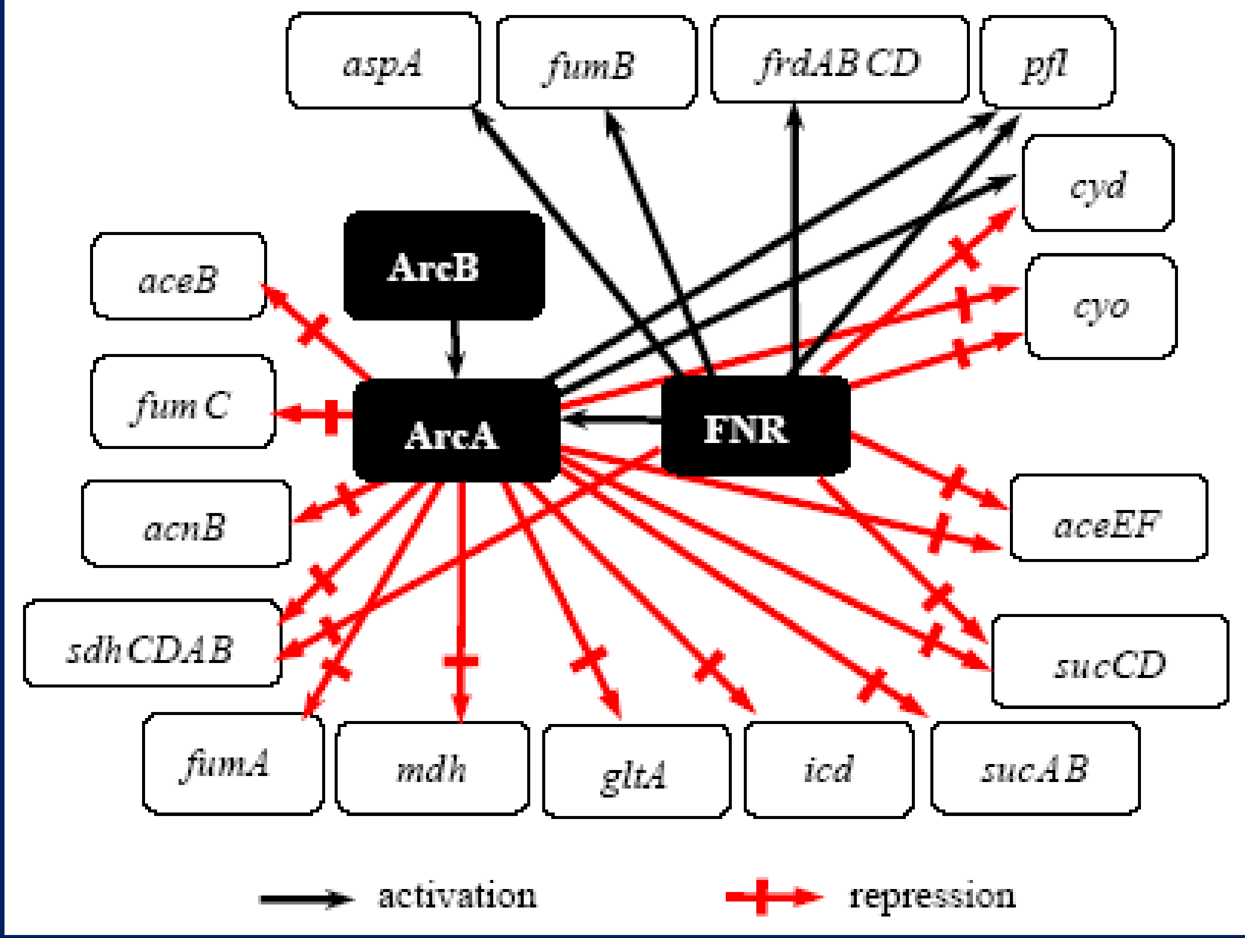
σ^{32} is rapidly degraded but when cells experience a heat shock, this degradation process is inhibited.

↓

More sigma factor.

More RNA polymerase directed to more heat shock promoters.

[oxygen]



A group of global regulators includes

- the one component fumarate & nitrate reduction (FNR) protein, and
- the two-component anoxic redox control (Arc) system

Fnr Modulon

Fnr is a global transcription regulator; it has 4 Cys residues that have been shown to bind a 4Fe-4S cluster under some conditions and 2 [2Fe-2S] centers under other conditions.
Hence, a redox-sensitive conformational change can occur that mediates control of transcription of ~30 transcription units and >70 genes.

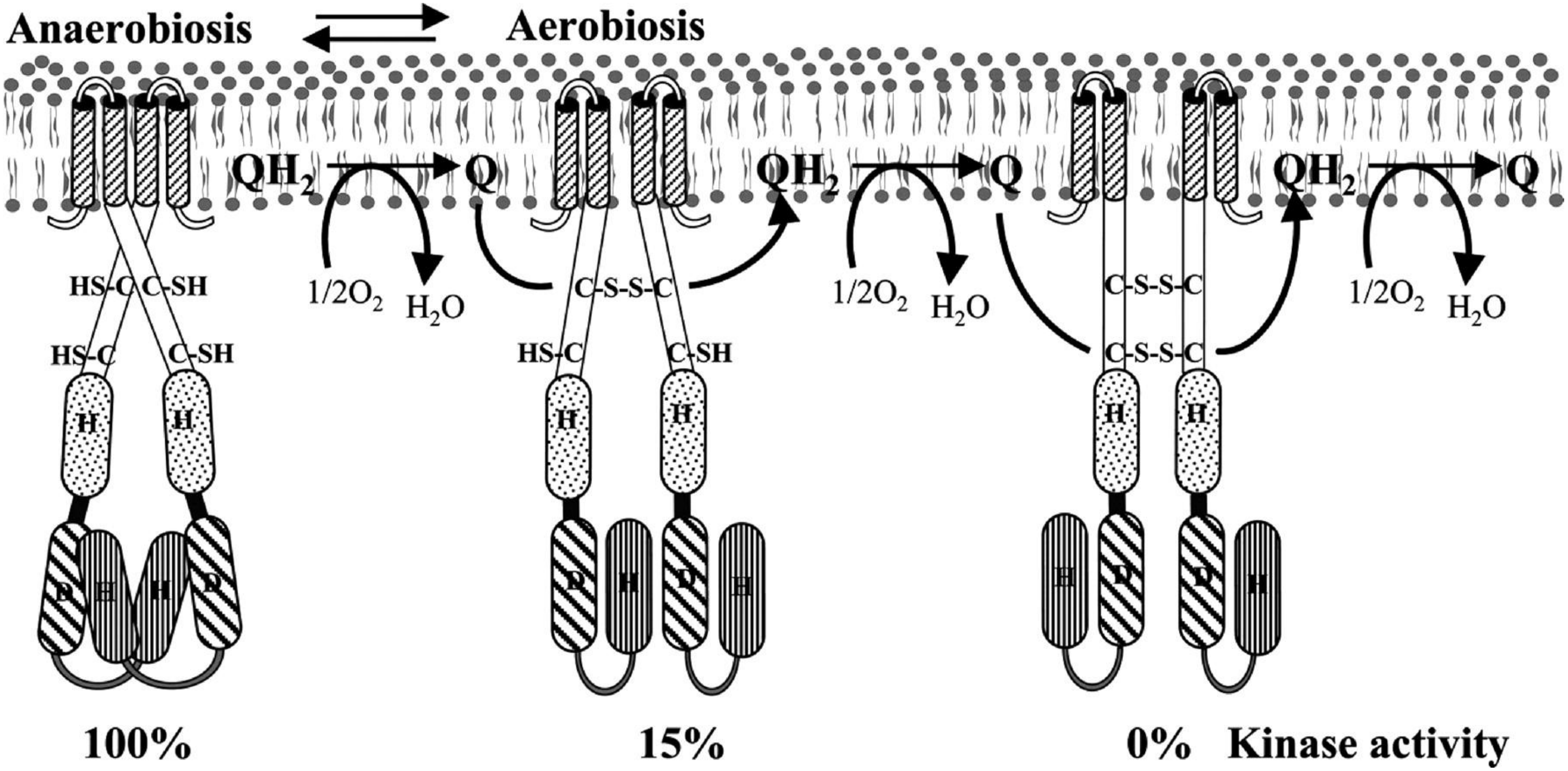
ArcA/B Modulon

In this system, ArcB is the sensor kinase, and is a membrane-bound protein found in the cytoplasmic membrane. **ArcA is a DNA-binding transcriptional regulator.**

Target genes include those encoding quinol oxidase, succinate dehydrogenase, superoxide dismutase and many others involved in aerobic metabolism and energy production.

With the initial onset of anaerobiosis ArcA is activated, and if these conditions persist or become more anaerobic, FNR is activated leading in turn to the upregulation of ArcA and amplification of its effect

Model for the redox regulation of the ArcB sensor kinase of E. coli through inactivation by oxygen.



Mascher T et al. Microbiol. Mol. Biol. Rev. 2006;70:910-938

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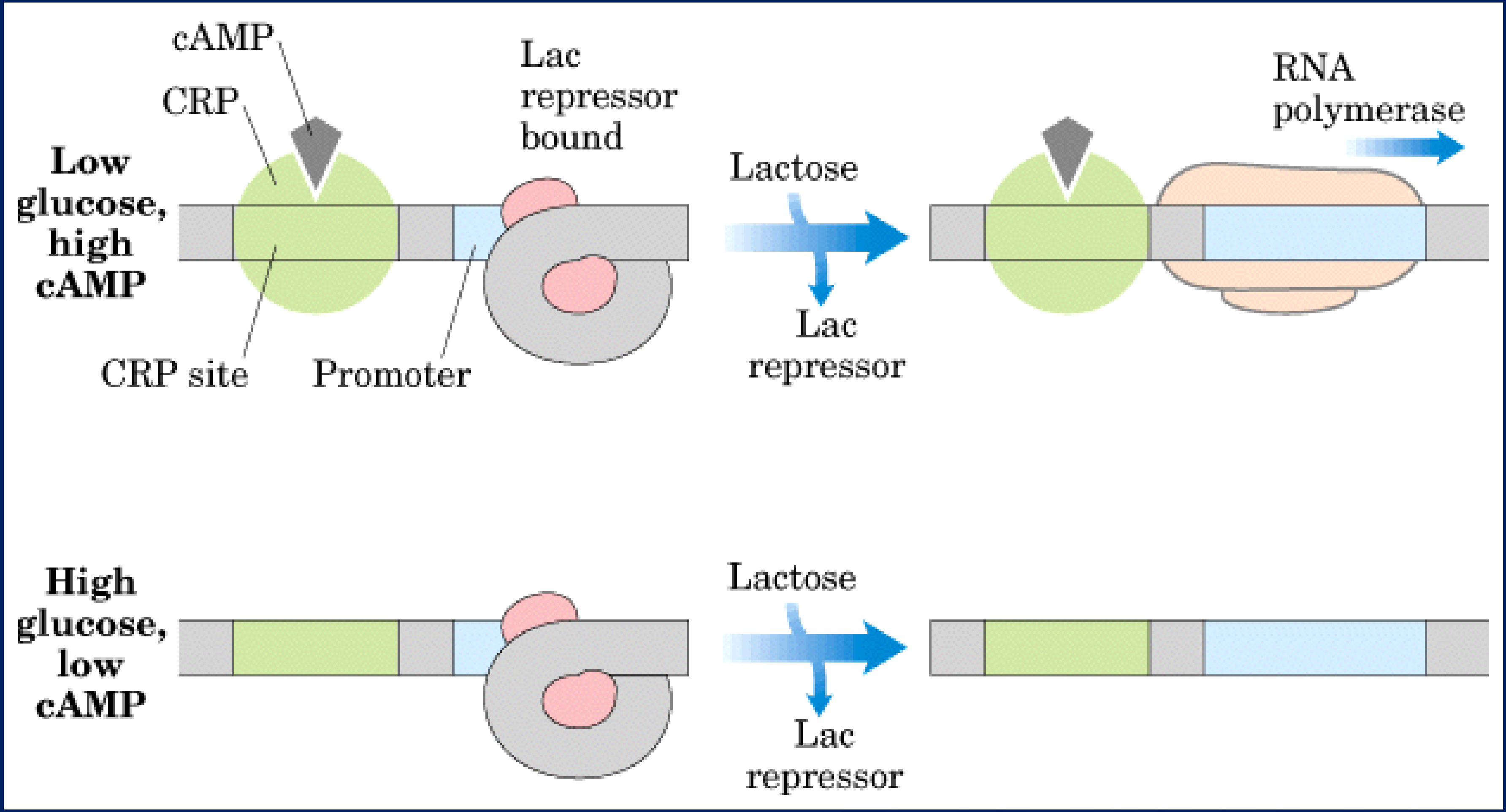
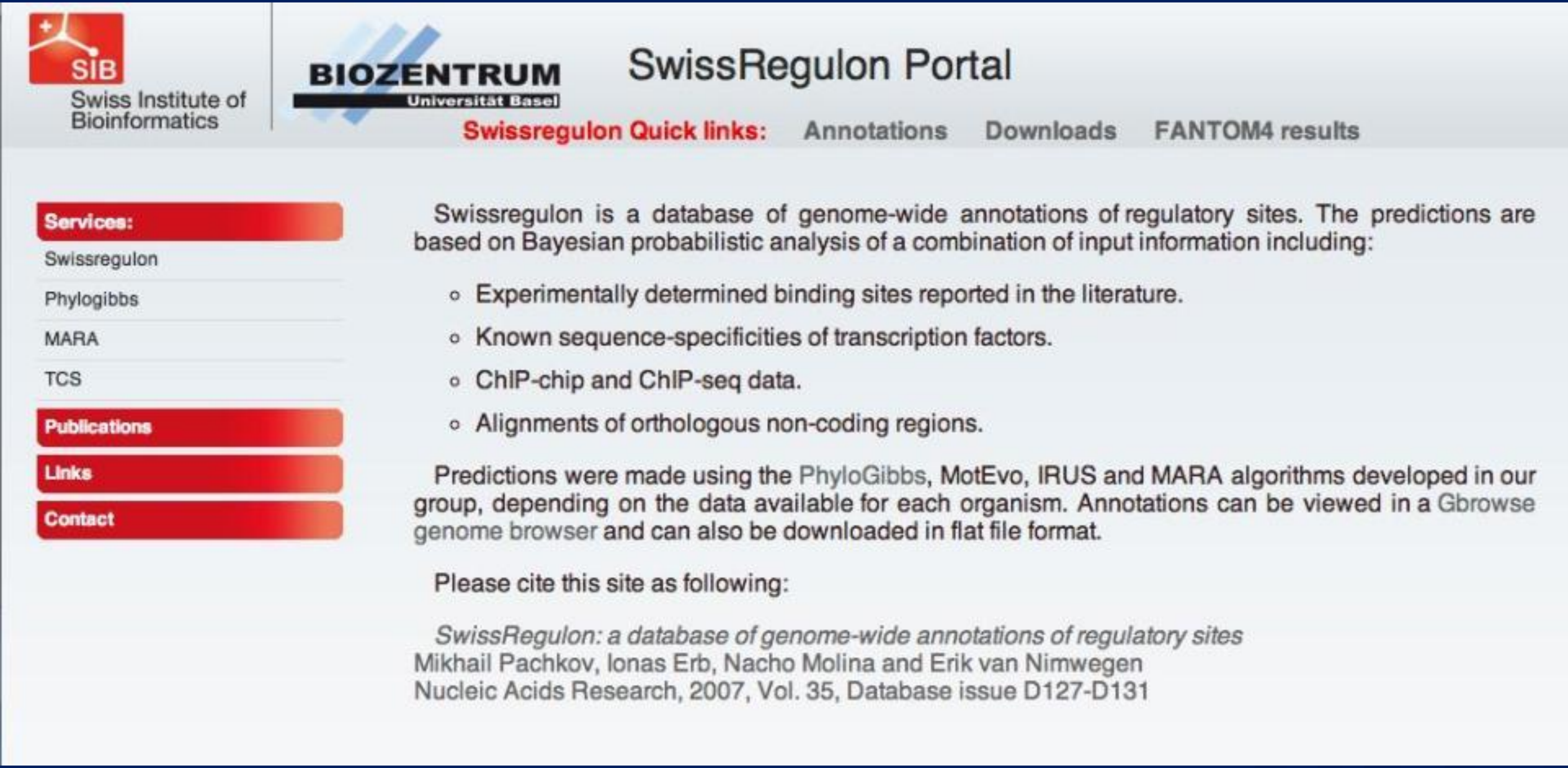
[glucose/other sugar]

- **Catabolite repression**: the effect of glucose concentration on the transcriptional regulation of the *lac* operon



Swiss Regulon

http://www.swissregulon.unibas.ch/cgi-bin/regulon



Swissregulon is a database of genome-wide annotations of regulatory sites. The predictions are based on Bayesian probabilistic analysis of a combination of input information including:

- Experimentally determined binding sites reported in the literature.
- Known sequence-specificities of transcription factors.
- ChIP-chip and ChIP-seq data.
- Alignments of orthologous non-coding regions.

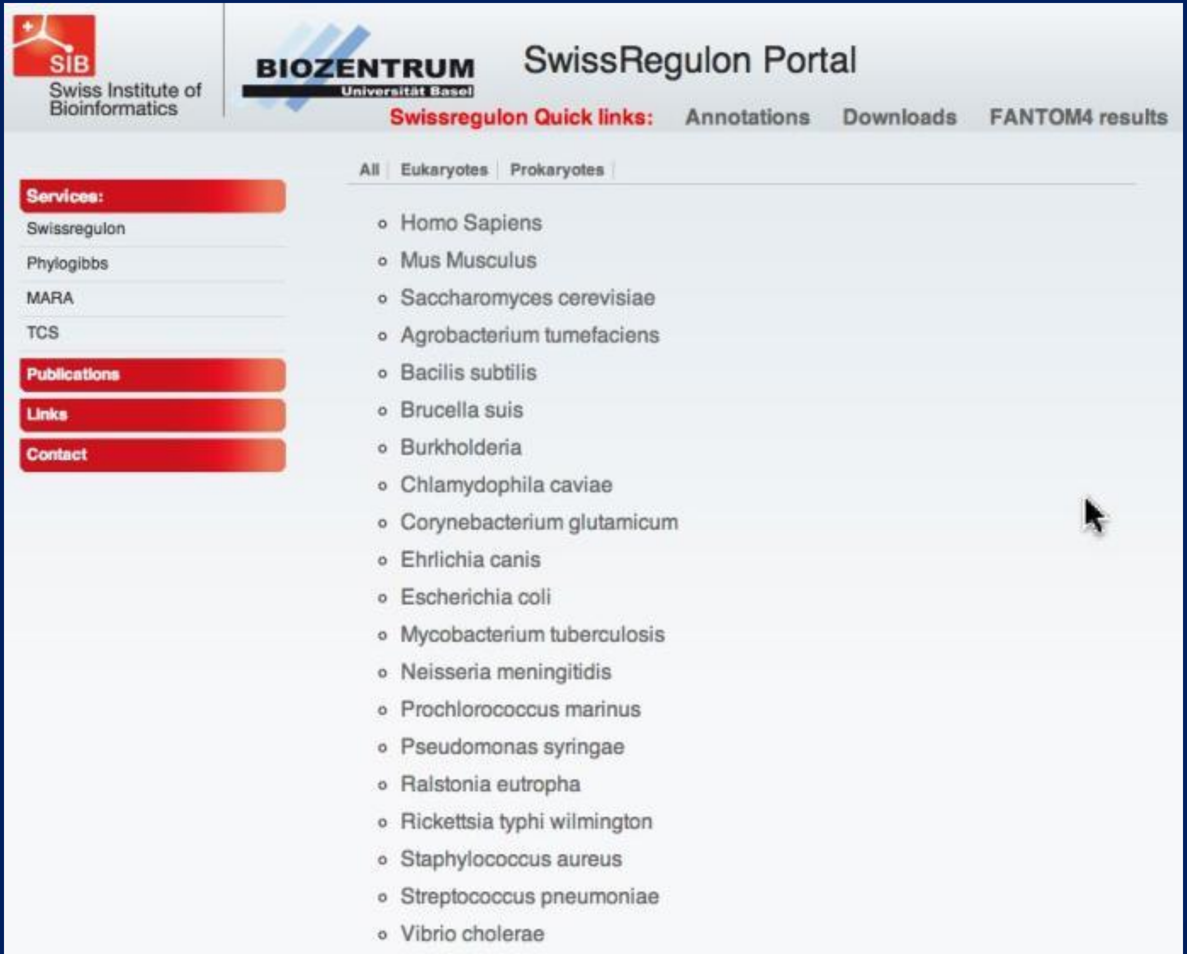
Predictions were made using the PhyloGibbs, MotEvo, IRUS and MARA algorithms developed in our group, depending on the data available for each organism. Annotations can be viewed in a Gbrowse genome browser and can also be downloaded in flat file format.

Please cite this site as following:

SwissRegulon: a database of genome-wide annotations of regulatory sites
Mikhail Pachkov, Ionas Erb, Nacho Molina and Erik van Nimwegen
Nucleic Acids Research, 2007, Vol. 35, Database issue D127-D131

Swiss Regulon Organisms

http://www.swissregulon.unibas.ch/cgi-bin/regulon



SIB Swiss Institute of Bioinformatics
BIOZENTRUM Universität Basel

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- Mus Musculus
- Saccharomyces cerevisiae
- Agrobacterium tumefaciens
- Bacilis subtilis
- Brucella suis
- Burkholderia
- Chlamydophila caviae
- Corynebacterium glutamicum
- Ehrlichia canis
- Escherichia coli
- Mycobacterium tuberculosis
- Neisseria meningitidis
- Prochlorococcus marinus
- Pseudomonas syringae
- Ralstonia eutropha
- Rickettsia typhi wilmington
- Staphylococcus aureus
- Streptococcus pneumoniae
- Vibrio cholerae

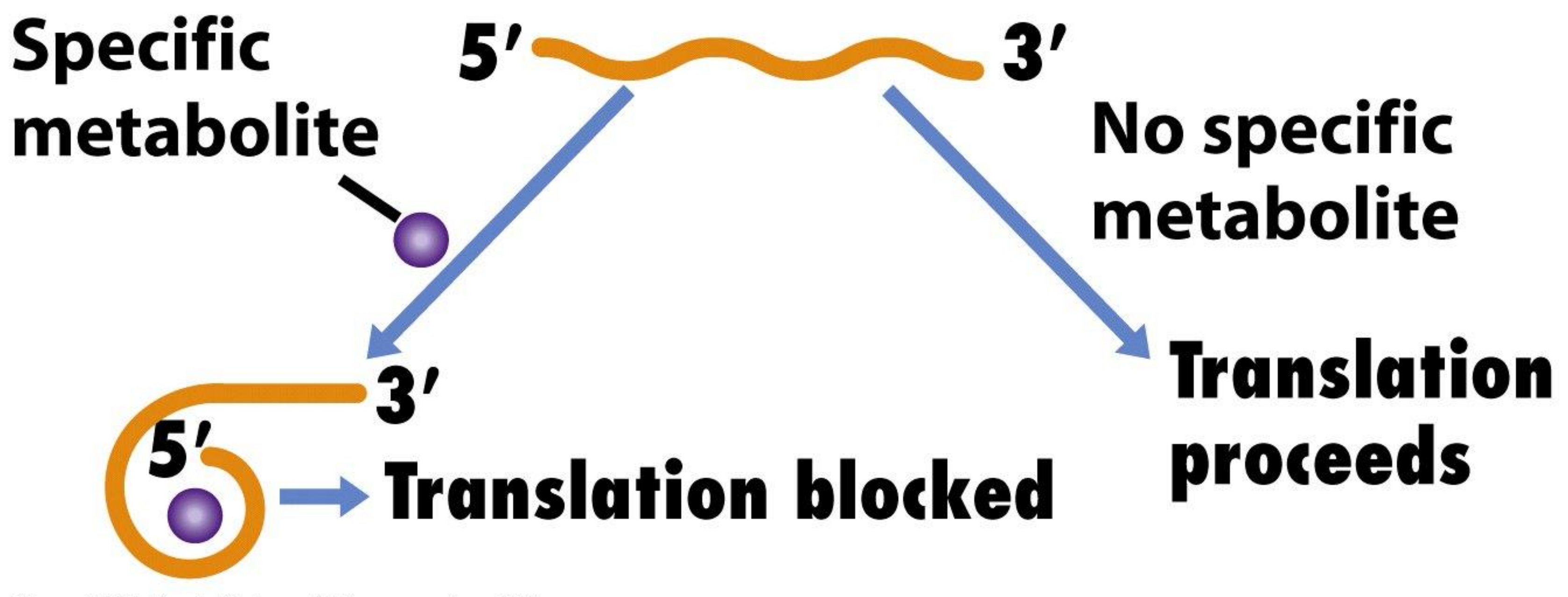
Other Mechanisms of Regulation: Attenuation

- **Attenuation** is a mechanism whereby gene expression (typically at the level of transcription) is controlled ***after initiation*** *of RNA synthesis*

- Attenuation mechanisms involve a coupling of transcription and translation and can therefore occur **only in prokaryotes**.

- A unique form of small RNAs are the ***riboswitches***. These are mRNAs that contain sequences upstream of the coding sequences. A **riboswitch** activity is controlled by the metabolite product or another small ligand.

Metabolite binding effects secondary structure of RNA



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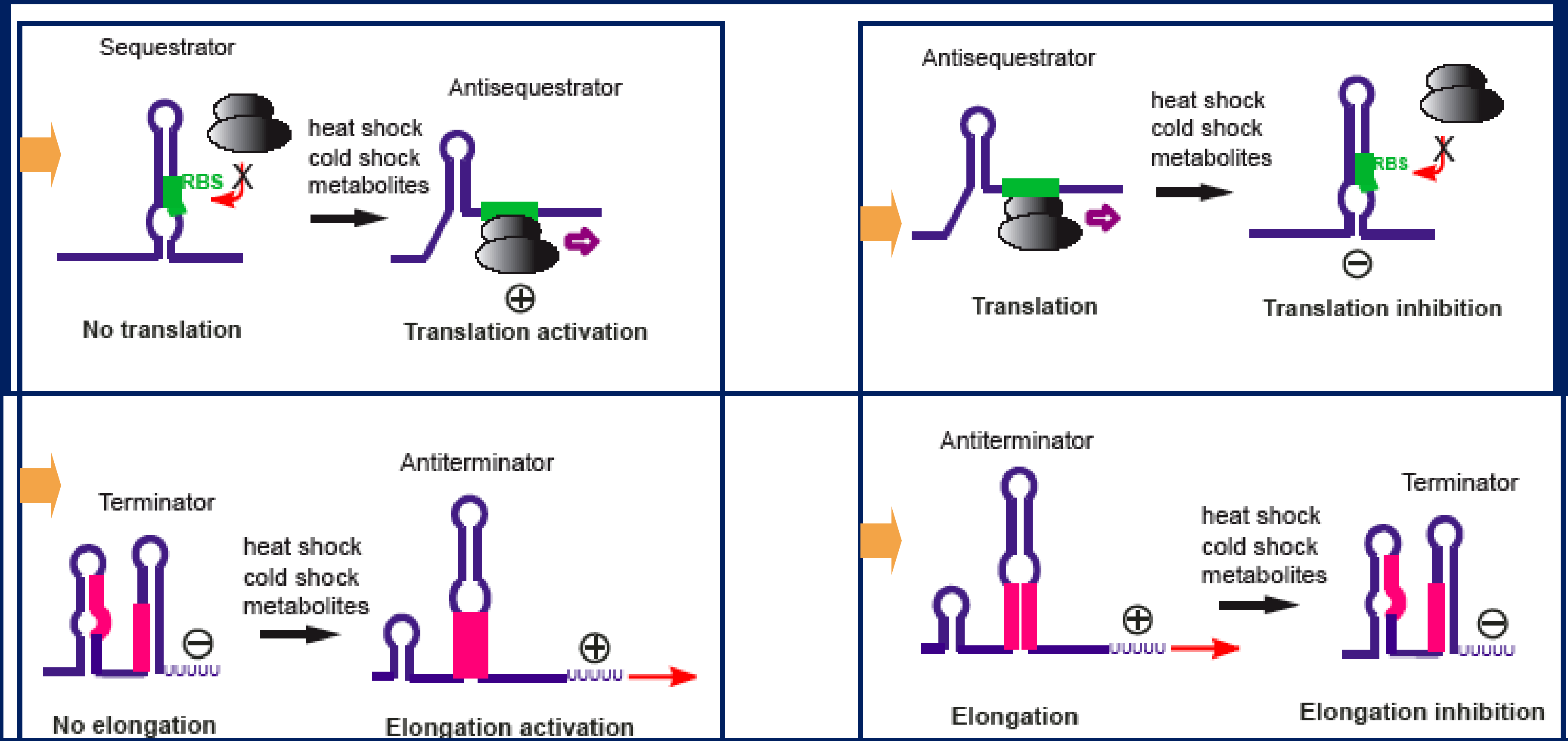
- **Aptamer –** An RNA domain that binds a small molecule; this can result in a conformation change in the RNA.

Sensory mRNAs - Riboswitches (alternative conformers…)

Different steps of translation: initiation and elongation

Activation

Repression



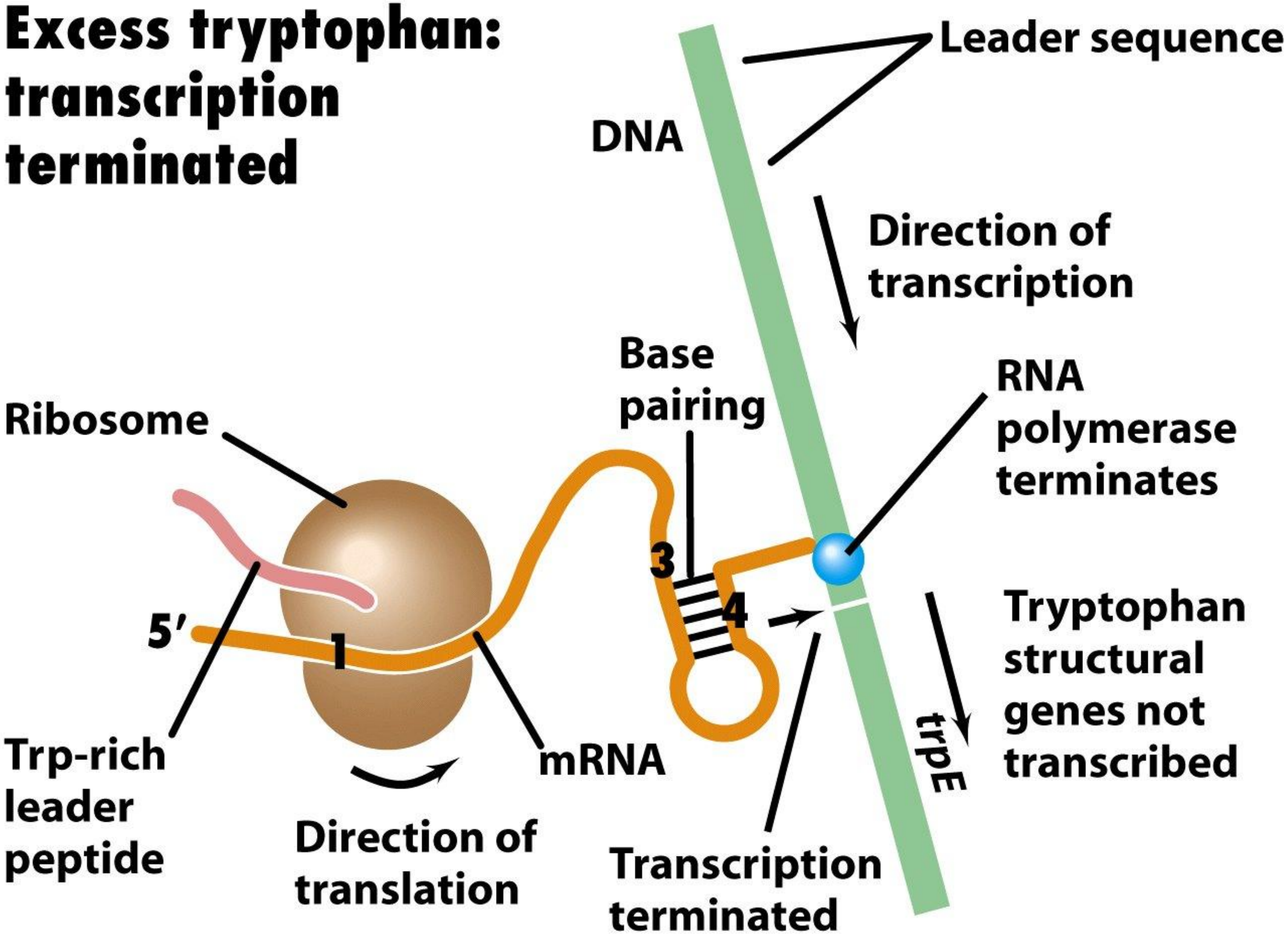
Narberhaus F. (2002) Arch. Microbio. 178, 404
Mandal M. & Breaker R. (2003) Cell 113, 577
Nudler E. & Mironov A. (2004) TBS 29, 11

Sequestrate= наложить арест

RBS=ribosome-binding site

Self-induced mRNA degradation

Excess tryptophan: transcription terminated



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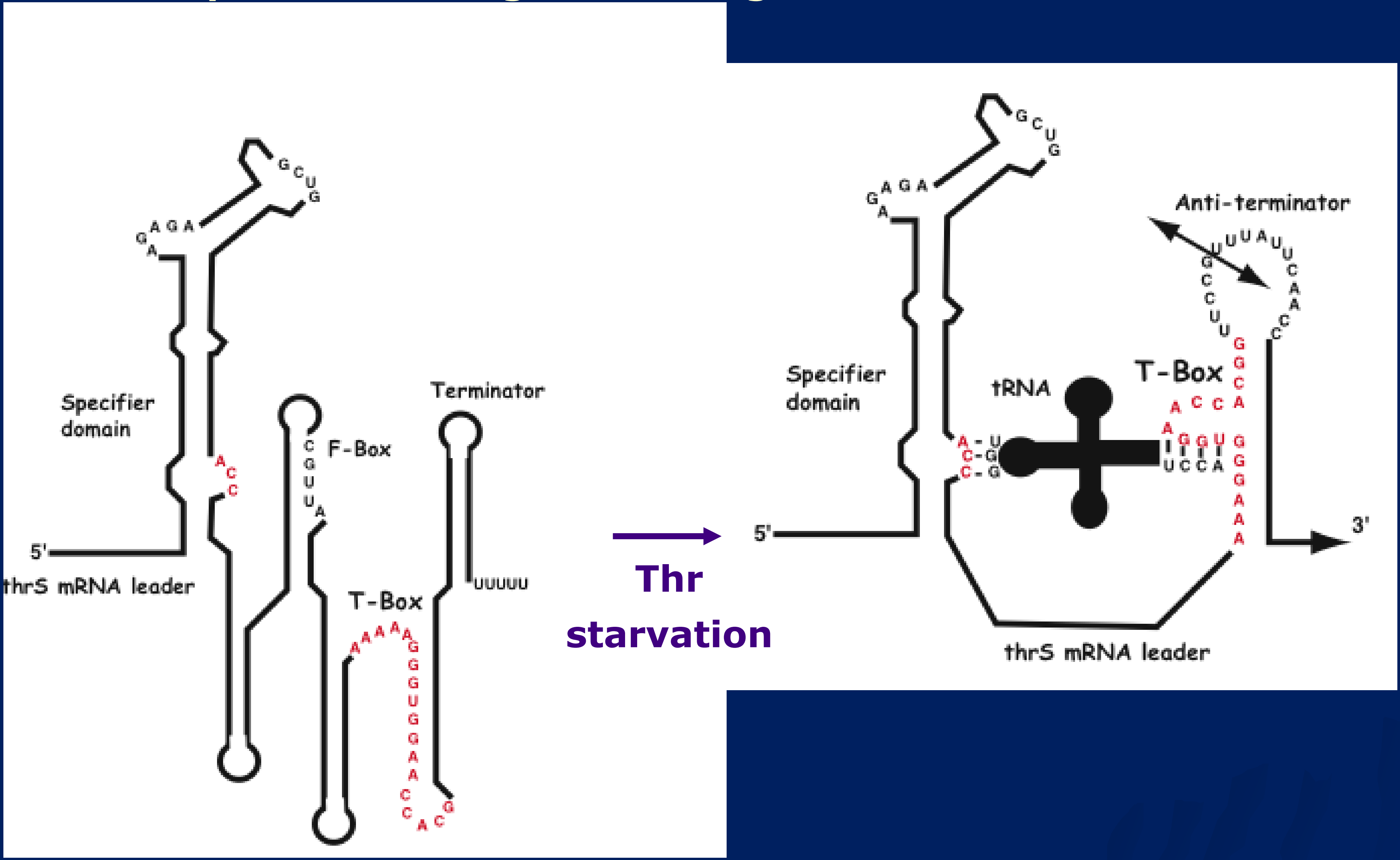
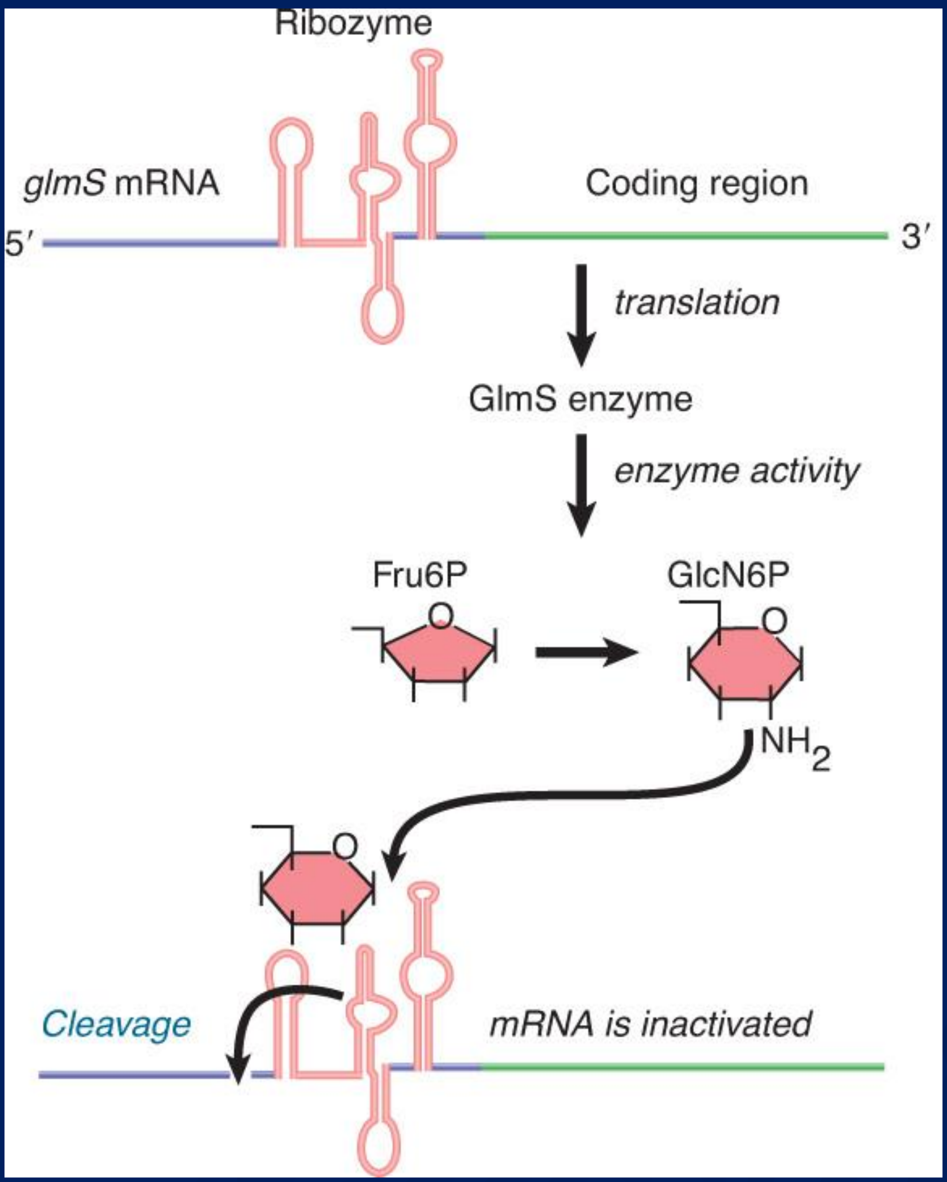
B. Subtilis uncharged tRNA induced antitermination of transcription of the cognate aaRS gene



Putzer et al. 2002 NARes. 30:3026 and ref therein

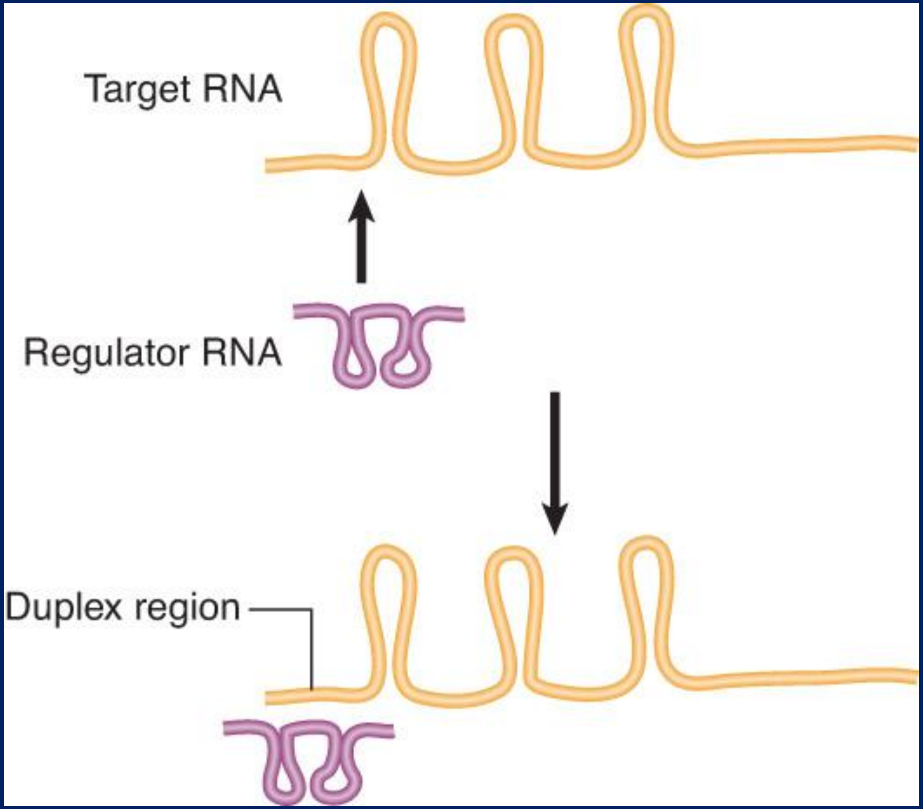
A riboswitch may be a **ribozyme**

- GlcN6P activates a ribozyme that cleaves the mRNA



Regulatory RNAs

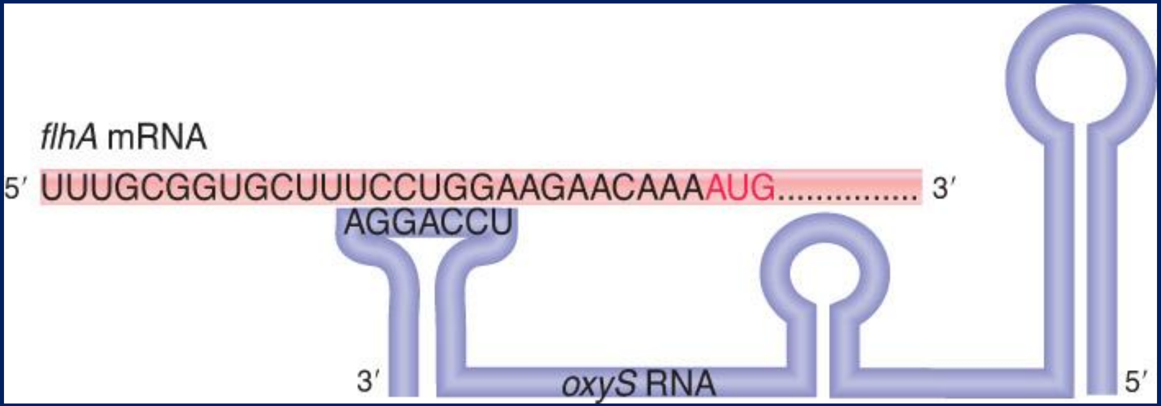
- RNA functions as a regulator by forming a region of secondary structure (either inter- or intramolecular) that changes the properties of a target sequence.



Regulator RNA binds RNA target

Bacteria Contain Regulator RNAs

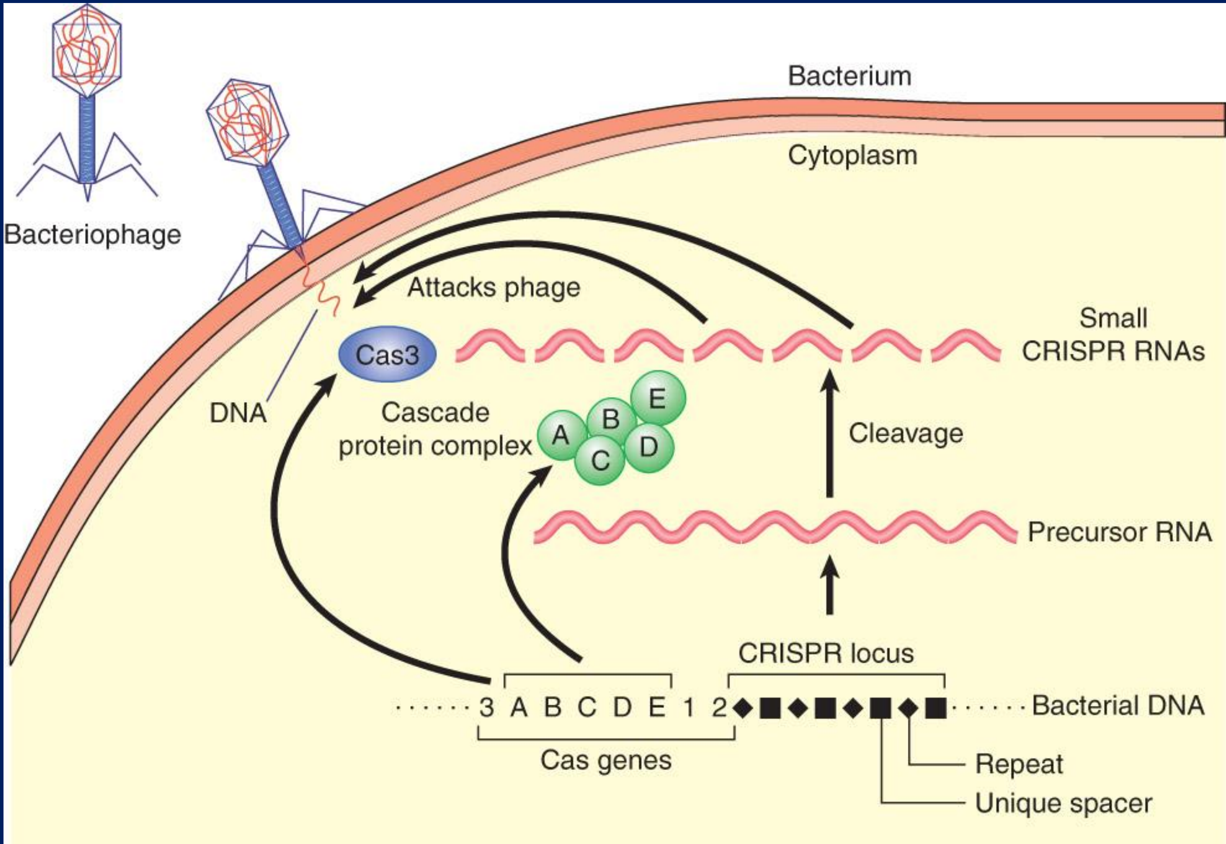
- Bacterial regulator RNAs are called **sRNAs**.
- Several of the sRNAs are bound by the protein Hfq, which increases their effectiveness.



- A 3’ terminal loop in oxyS RNA pairs with the initiation site of flhA mRNA
- The *oxyS* sRNA activates or represses expression of >10 loci at the posttranscriptional level.
- oxyS is controlled by oxyR and H_2O_2

Bacteria Contain Regulator RNAs

- Tandem repeats can be transcribed into powerful antiviral RNAs
- **CRISPR –** *C*lusters of *r*egularly *i*nterspersed *s*hort *p*alindromic *r*epeats in prokaryotes that are transcribed and processed into short RNAs that function in RNA interference.



The CRISPR locus in *E. coli* is transcribed into a larger precursor RNA

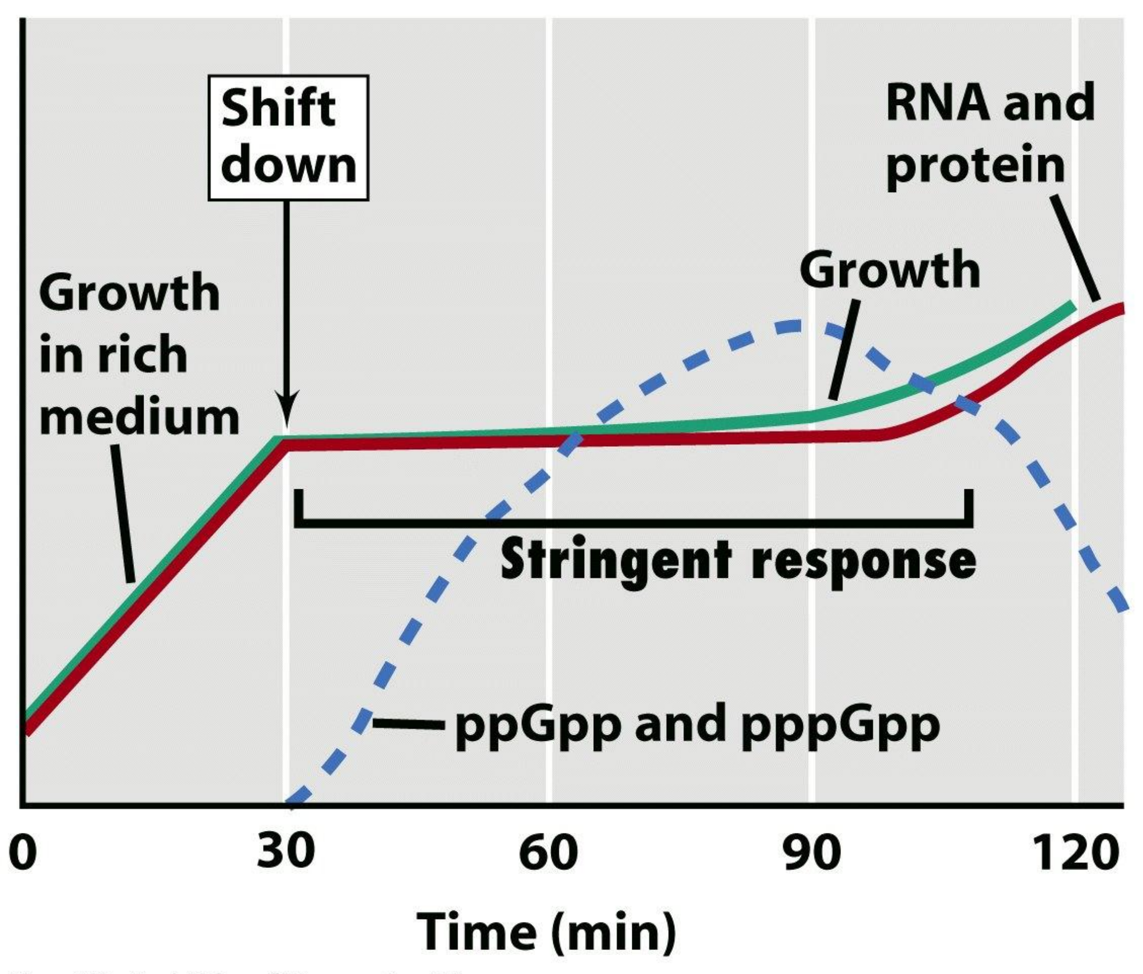
Chromosomally encoded sRNAs - biological roles

sRNAs		target(s)	encodes	response/ biological role
MicF	$\xrightarrow{\ominus}$	ompF mRNA	porin	membrane stress
MicC	$\xrightarrow{\ominus}$	ompC mRNA	porin	membrane stress
MicA	$\xrightarrow{\ominus}$	ompA mRNA	porin	membrane stress?
DsrA	$\xrightarrow{\ominus}$ $\searrow \oplus$	hns mRNA	transcriptional regulator	thermoregulation
RprA	$\xrightarrow{\oplus}$	rpoS mRNA	stress response σ^S	general stress
OxyS	$\nearrow$ $\xrightarrow{\ominus}$	fhlA mRNA	transcriptional activator	oxidative stress
RyhB	$\xrightarrow{\ominus}$	sodB mRNA etc	iron-storage proteins	iron homeostasis
IstR	$\xrightarrow{\ominus}$	tisAB mRNA	toxin	SOS response
RdlD	$\xrightarrow{\ominus}$	ldrD mRNA	killing peptide	purine metabolism?
GadY	$\xrightarrow{\oplus}$	gadX mRNA	transcriptional activator	acid stress
DicF	$\xrightarrow{\ominus}$	ftsZ mRNA	cell division protein	cell division
GcvB	$\xrightarrow{\ominus}$	oppA+dppA mRNA?	periplasmic BP	peptide transport
Spot42	$\xrightarrow{\ominus}$	galK mRNA etc	gal operon enzyme	sugar metabolism
6S RNA	$\xrightarrow{?}$	σ^{70}	Sigma factor	stationary phase survival
CsrB CsrC	$\xrightarrow{\ominus}$	CsrA protein	regulator	carbon metabolism, virulence

(*from Wagner EGH et al.*)

[amino acids]
The Stringent Response

- is a global control mechanism triggered by amino acid starvation



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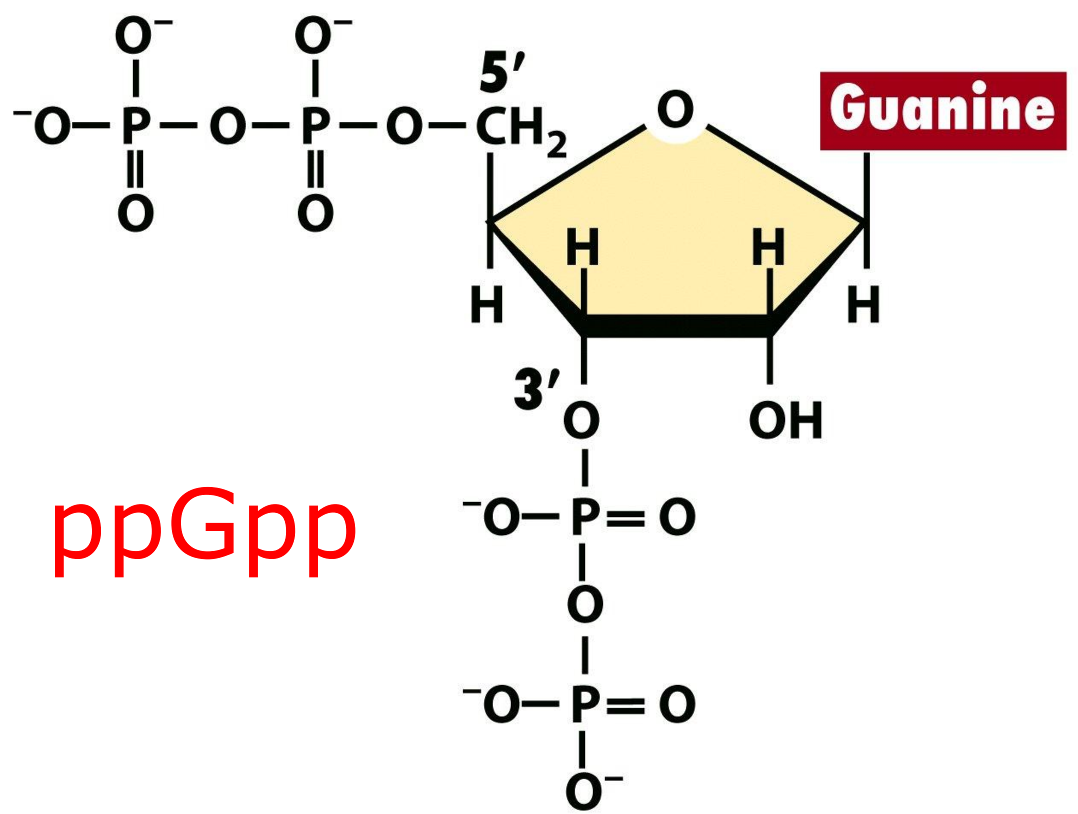
- The *alarmones* ppGpp and pppGpp are produced by RelA, a protein that monitors ribosome activity.



ppGpp

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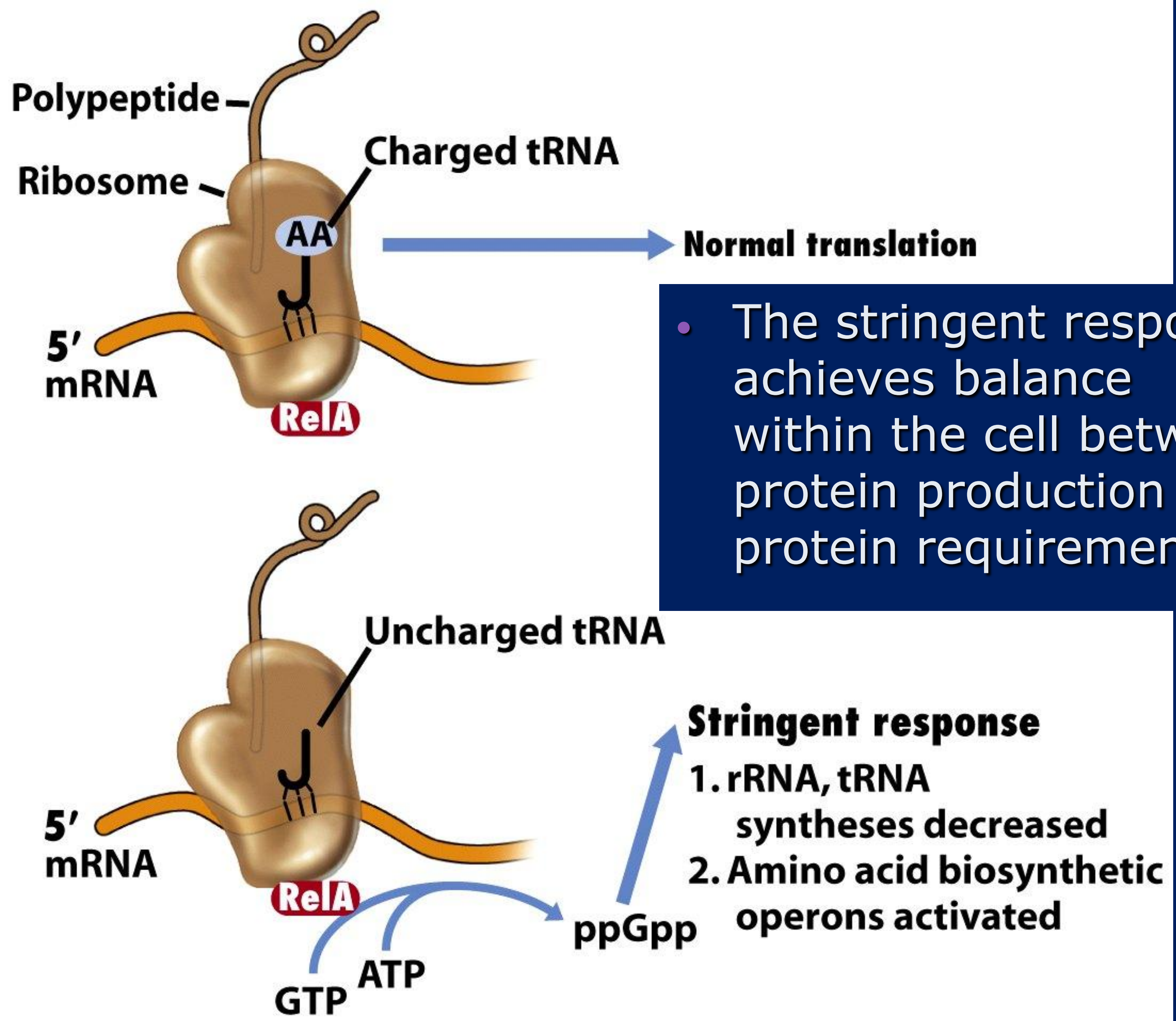

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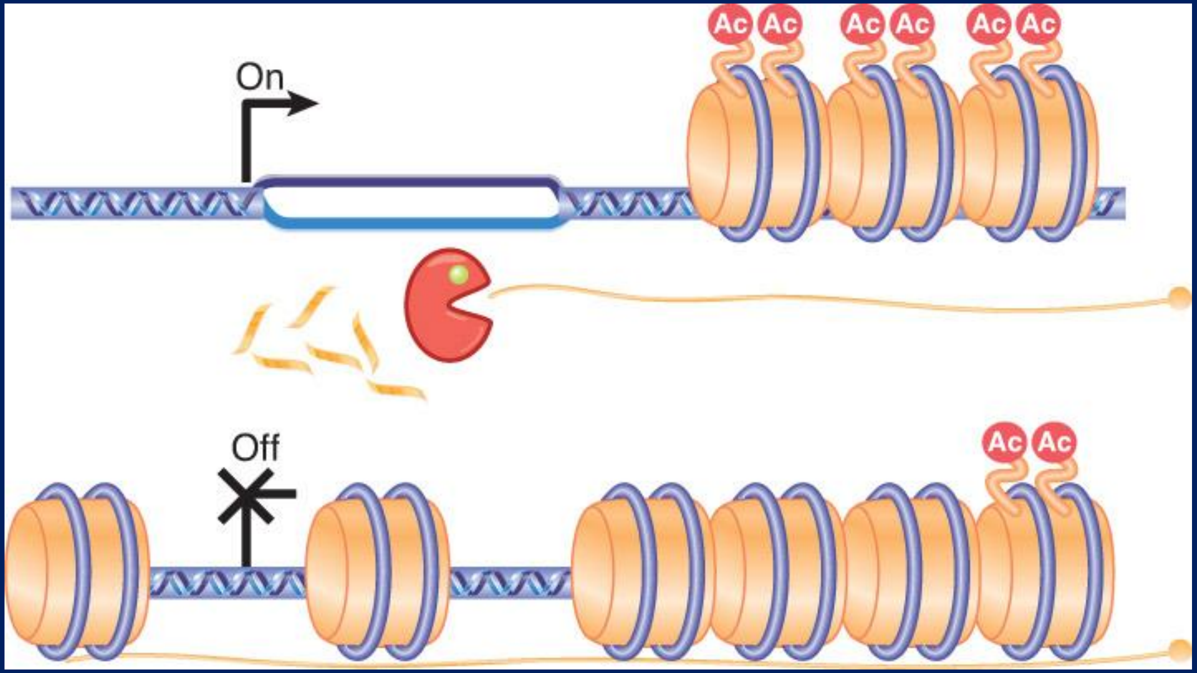
- The stringent response achieves balance within the cell between protein production and protein requirements.

Gene Regulatory Mechanisms

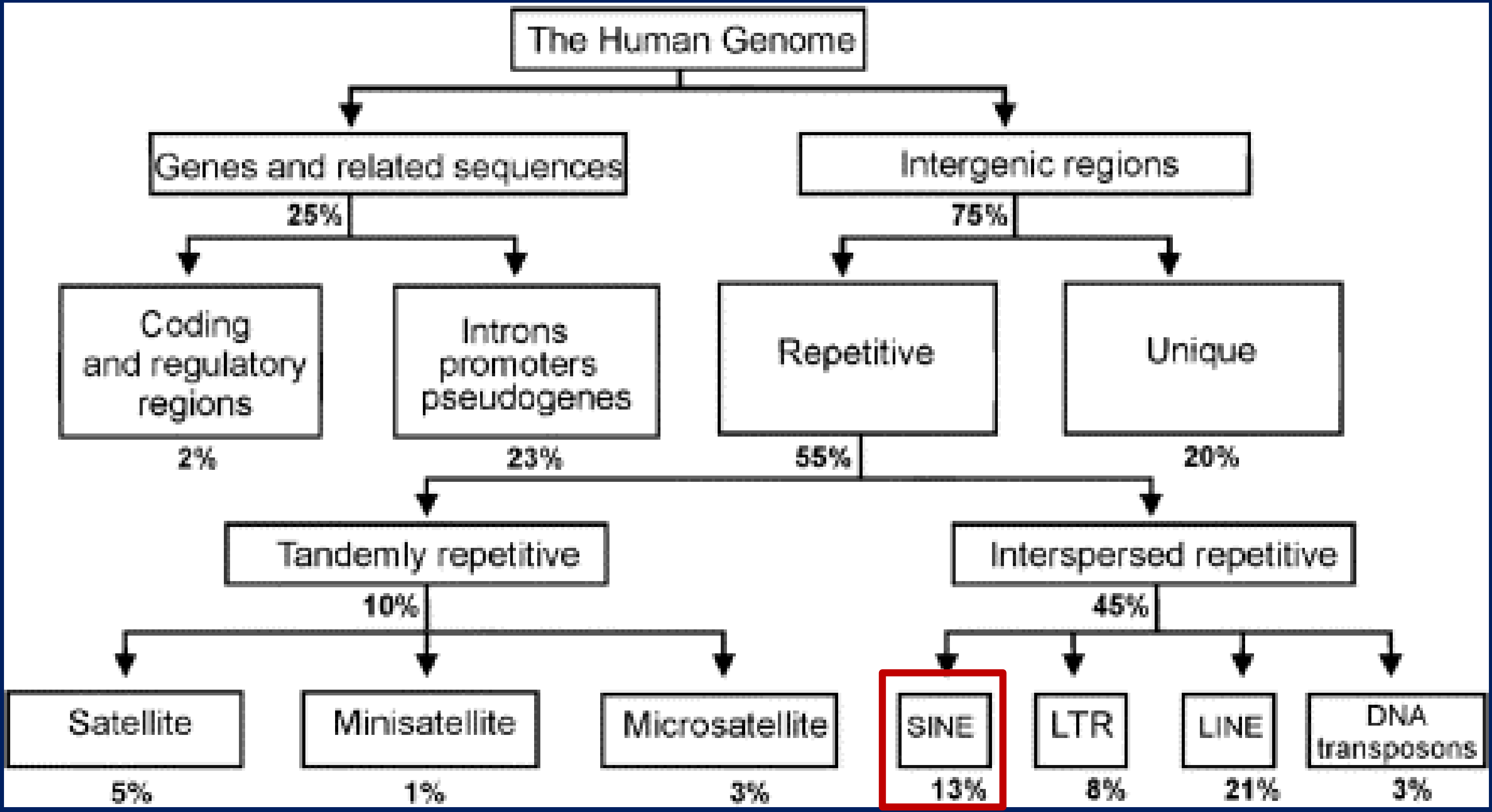
- **Transcriptional Mechanisms**
  - **Type of promoters & RNA polymerase (pro- + eukaryotes)**
  - **Control of Transcription (Constitutive / Inducible/ Repressible)**
  - **Transcription Factors and TFBS**
  - **Transcription attenuation → riboswitches (only prokaryotes)**
- **Epigenetic Mechanisms (only eukaryotes)**
  - **DNA methylation at CpG sites**
  - **Histone acetylation**
  - **Chromatin remodeling**
- **RNA processing (only eukaryotes)**
  - **Capping**
  - **Splicing and Alternative Splicing**
  - **Poly-Adenylation**
  - **RNA degradation rates**
  - **RNA export to cytoplasm**
- **Translation inhibition and mRNA degradation (only eukaryotes)**
  - **MicroRNAs and small interfering RNAs (siRNAs or RNAi) degrading mRNA**

REGULATION OF METABOLISM AND GENE EXPRESSION IN EUKARYA

Genome structure in eukarya



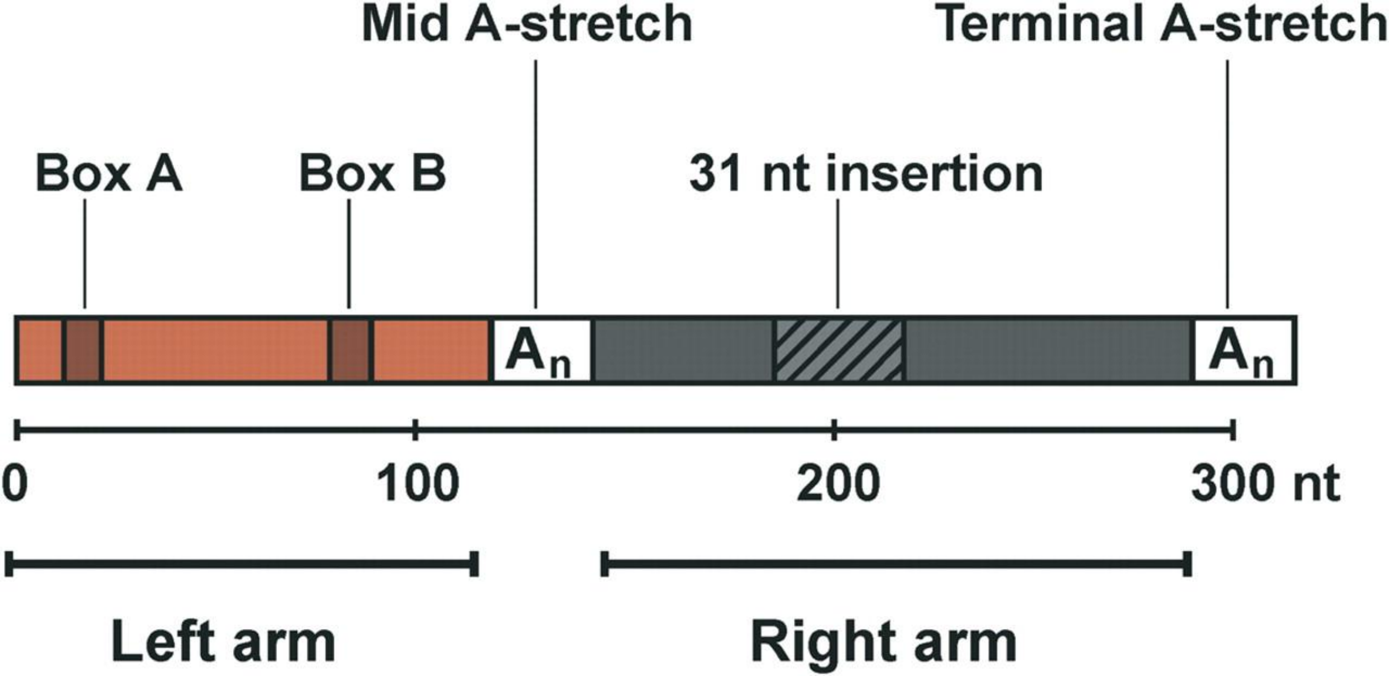
Composition of the human genome



- The Human Genome
  - Genes and related sequences 25%
    - Coding and regulatory regions 2%
    - Introns promoters pseudogenes 23%
  - Intergenic regions 75%
    - Repetitive 55%
      - Tandemly repetitive 10%
        - Satellite 5%
        - Minisatellite 1%
        - Microsatellite 3%
      - Interspersed repetitive 45%
        - SINE 13%
        - LTR 8%
        - LINE 21%
        - DNA transposons 3%
    - Unique 20%

Repetitive sequences that shape the human transcriptome.
A Jasinska and WJ Krzyzosiak
FEBS Lett, June 1, 2004; 567(1): 136-41

Architecture of Alu elements



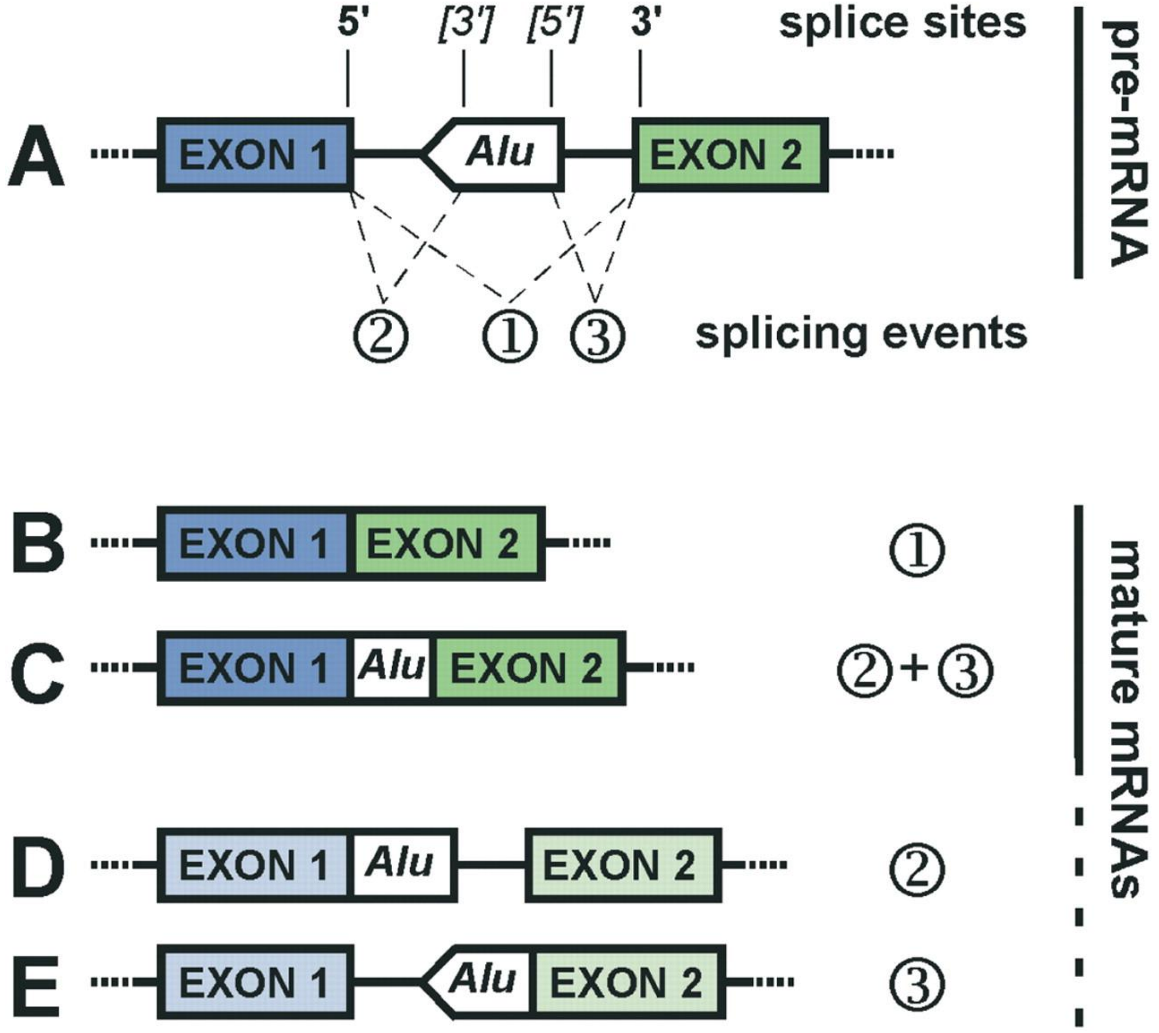
Hasler, J. et al. Nucl. Acids Res. 2006 34:5491-5497

Potential functional roles of ALUs in introns

- Up to 33% of the total number of **CpG sites** in the genome are found within SINE.
  Alus have been reported to be **highly methylated** in most somatic tissues.
  Alus demethylation occurs in aging and cancer and is associated with gene reactivation ad genomic instability [Rodriguez et al, 2008]

- Alus are carriers of **different TF binding sites** (nuclear factors, hormones, calcium nuclear factors and others) [by Polak, Domany, 2006]

Exonization of intronic Alu elements



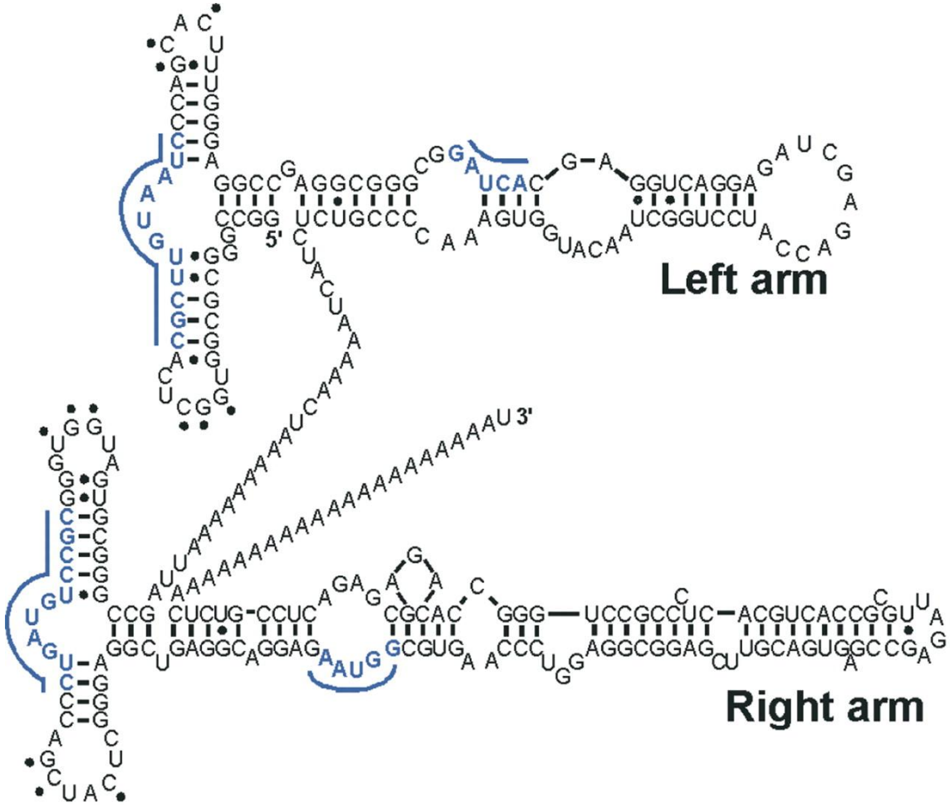
Hasler, J. et al. Nucl. Acids Res. 2006 34:5491-5497

Potential functional roles of exonized ALUs

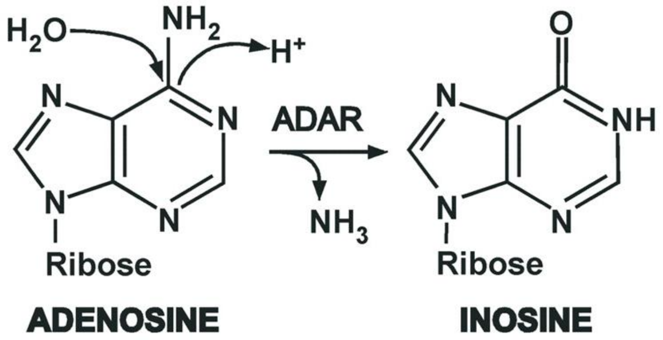
→**Transcriptome diversity and versatility**

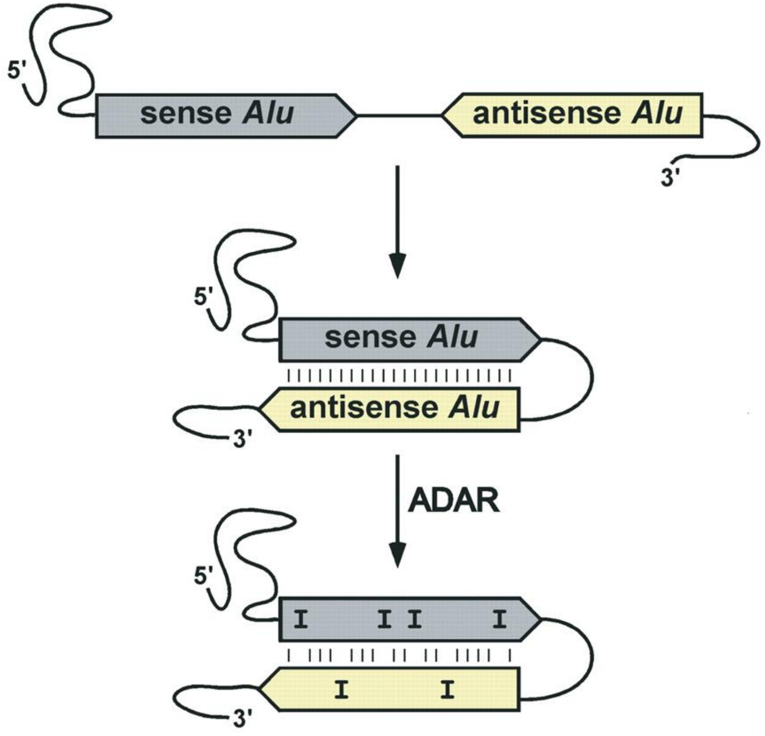
- All Alu-derived exons were found to be **alternatively spliced** [Sorek et al, 2002]. Alus are present in one or several (but not all) splice isoforms.
- Alternative mRNAs **polyadenylation** within exonized Alus lead to the expression of transcripts with different UTR in different tissues [Chen et al, 2009].
- Transcripts with Alus undergo adenosine-to-inosine (A-to-I) **RNA editing** in multiple sites [Barak et al, 2009].

AluRNA secondary structure



A-to-I editing in Alu elements





Hasler, J. et al. Nucl. Acids Res. 2006 34:5491-5497

Potential functional roles of exonized ALUs

→**Antisense-mediated control of gene expression (miRNA targets)**

- The majority of the Alus **in 3'UTRs** of analyzed human genes were found to carry strong potential **target sites for different miRNAs** [Smallheiser, 2006], [Daskalova et al, 2006].
- Alus contributed to growth of cluster of **miRNAs** that are predicted to **target free Alu transcripts** and to prevent catastrophic or self-destructive intensities of Alu retroposition. So co-evolution between Human MicroRNAs and Alu-Repeats could occur [Lehnert, 2009]

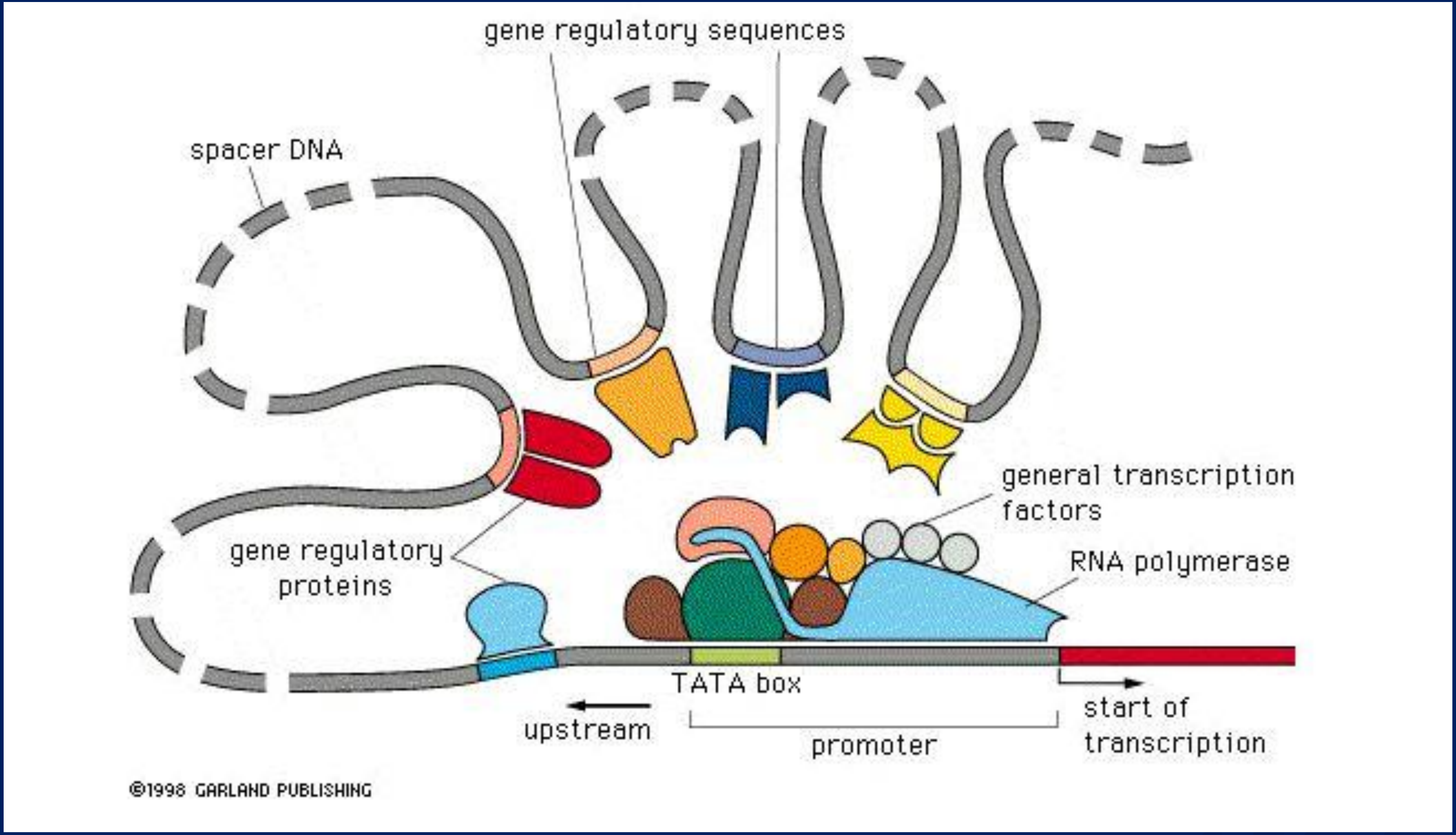
Upstream Regulatory Sequences

- Promoter (general term)

- UAS (Upstream Activation Sequence)
- Enhancers

- URS (Upstream Repression Sequence)
- Operator

Multiple Enhancer Sequences



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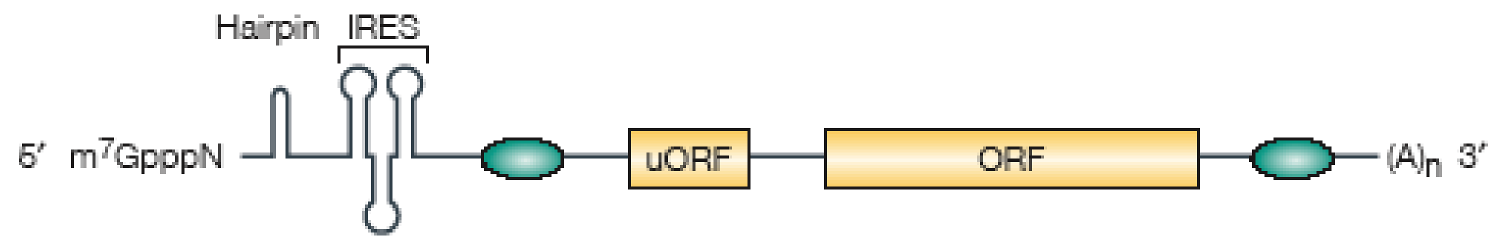
Elements that influence translation of eukaryotic mRNA



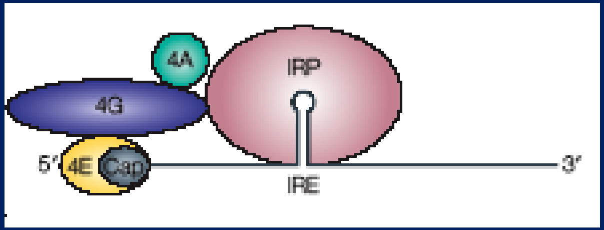
- ○ **cap structure and the polyA tails : canonical motifs**
- ○ **Secondary structures close to the 5’ end block translation initiation**
- ○ **IRES: ribosome entry site mediates cap-independant translation, shunt mechanism…**
- ○ **short ORF reduced translation of the main ORF**
- ○ **binding sites for trans-acting regulatory factors (protein, miRNA…)**

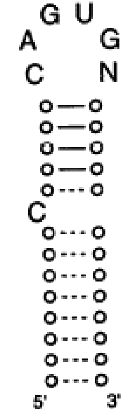
Eukaryotic post-transcriptional operons ?

One regulatory protein targets functionally related mRNAs

(Keene 2003 Mol Cell 12: 1347)

Iron regulatory protein



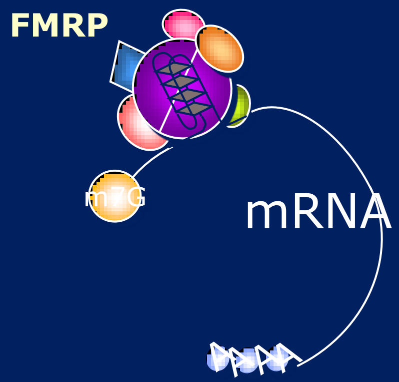


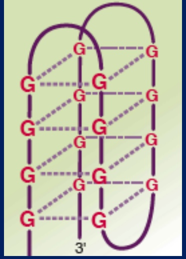
IRP and iron metabolism

- Ferritin
- Transferrin receptor
- e-ALAS
- Mitochondrial aconitase…

Dandekar & Hentze (1995 TIG 11:45)

Fragile X-syndrome Protein (associated in large mRNPs)





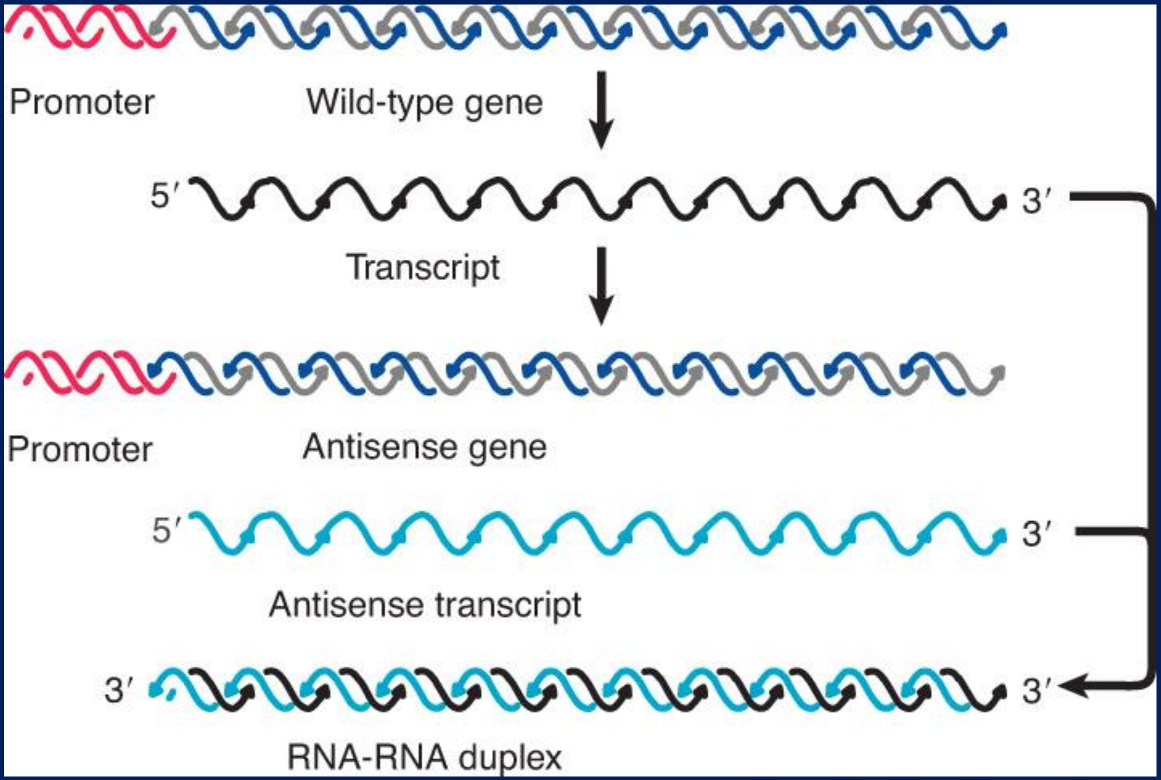
FMRP and neuronal function (actin cytoskeleton remodeling..

- FMR1*
- PP2Ac (Rac1 pathway)**
- MAP1b
- FGF-2***?…

*Schaeffer et al. 2001 EMBO J 20:4803
**Castets et al. 2005 Hum Mol Gen. 14:835
***Bonnal et al. 2003 JBC 278:39330
Darnell et al. 2001 Cell 107:489; 2005 Genes Dev

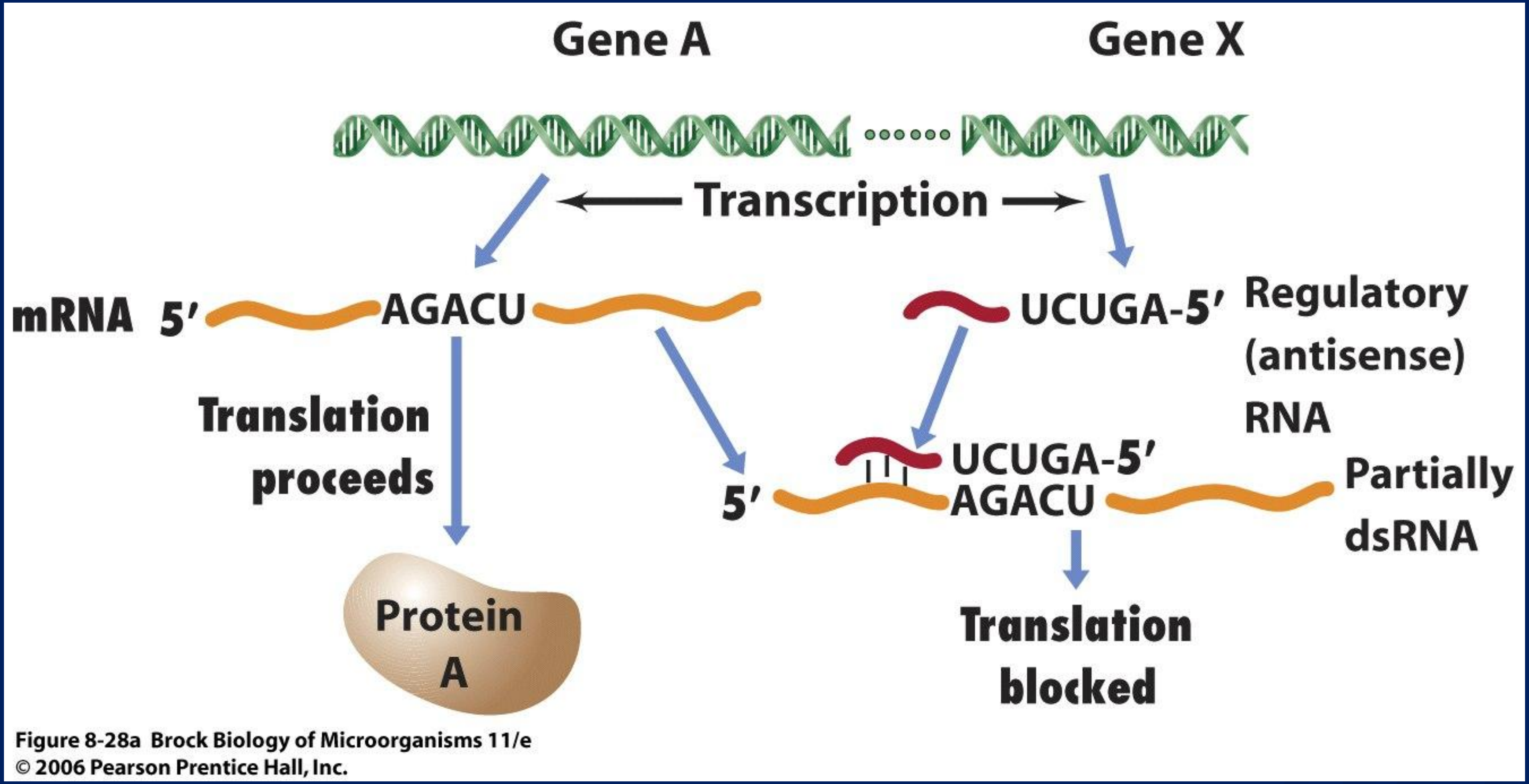
Noncoding RNAs Can Be Used to Regulate Gene Expression

- Vast tracts of the eukaryotic genome are transcribed.
- **antisense gene –** A gene that codes for an (antisense) RNA that has a complementary sequence to an RNA that is its target.
- **antisense RNA –** RNA that has a complementary sequence to an RNA that is its target.



Transcription of antisense RNA generates an RNA-RNA duplex

RNA interference



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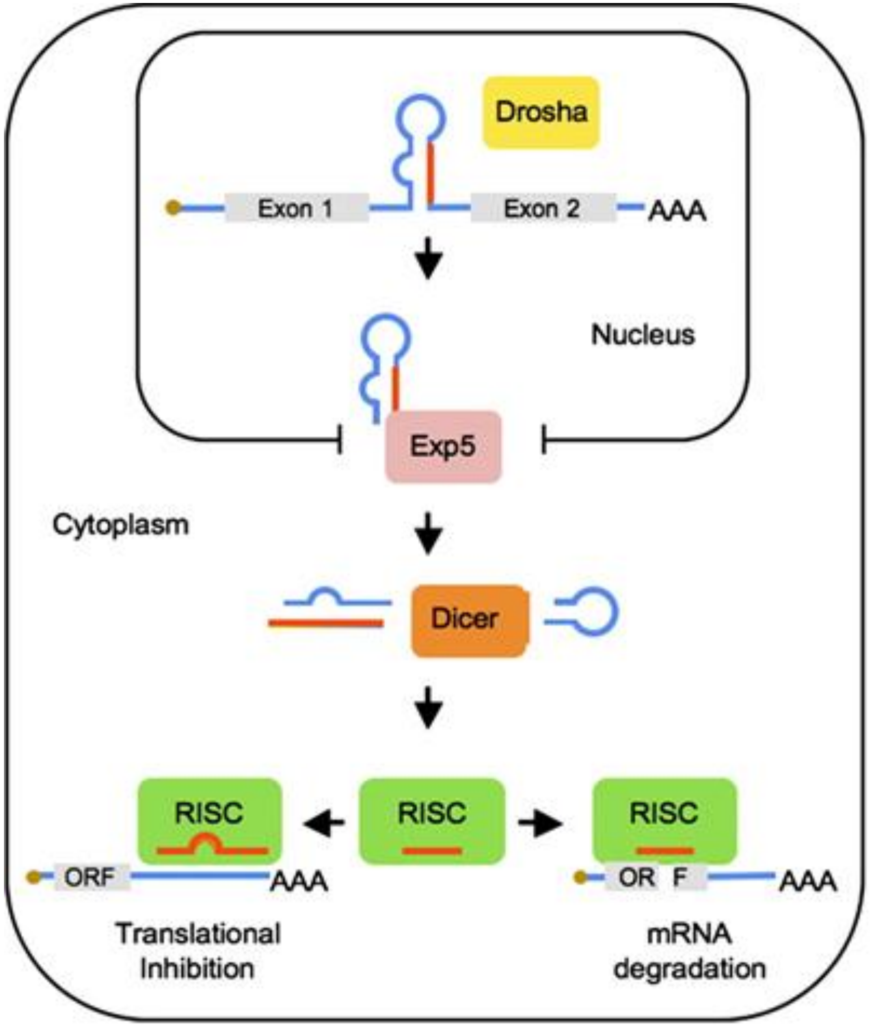
MicroRNAs Are Widespread Regulators in Eukaryotes

- Eukaryotic genomes code for many short (~22 base) RNA molecules called microRNAs (**miRNAs**).
- **RNA interference (RNAi) –** A process by which short (21 to 23) nucleotide antisense RNAs, derived from longer double-stranded RNAs, can modulate expression of mRNA by translation inhibition or degradation.
- **stRNA –** Short temporal RNA; a form of miRNA in eukaryotes that modulates mRNA expression during development.
- **piRNAs** regulate gene expression in germ cells and act to silence transposable elements.
- **siRNAs** are complementary to viruses and transposable elements.

Общая схема биогенеза микроРНК



Umbach J L , Cullen B R Genes Dev. 2009;23:1151-1164

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- гены микроРНК транскрибируются (с интрона, экзона или межгенного пространства) как длинные *pri*-mRNAs

- В созревании участвуют 2 РНКазы III - эндонуклеазы - *Drosha* и *Dicer*.

- После вырезания шпилькообразные (stem-loop) предшественники (60–70 nt) *pre*-miRNA экспортируются из ядра с помощью exportin-5.

- В цитоплазме *pre*-miRNA под действием Dicer превращается в зрелую ~22 nt miRNA, которая включается как одноцепочечная в RNA-induced silencing complex (RISC).

- МикроРНК направляют RISC на мРНК-мишени за счет частичного или полного комплементарного связывания

- Результат: репрессия трансляции или деградация мРНК-мишени

MicroRNAs Are Widespread Regulators in Eukaryotes

- **Drosha –** An endonuclease that processes double-stranded primary RNAs into short, ~70 base pair precursors for Dicer processing.

- **Dicer –** An endonuclease that processes double-stranded precursor RNA to 21 to 23 nucleotide RNAi molecules.

- **RISC –** RNA-induced silencing complex, a ribonucleoprotein particle composed of a short, single-stranded siRNA and a nuclease that cleaves mRNAs complementary to the siRNA.
  - It receives siRNA from Dicer and delivers it to the mRNA.

miRNAs effects

- Inhibition of translation (elongation)
- Co-translational protein degradation
- Inhibition of subunit joining
- Competition for cap structure
- Deadenylation and decaping for mRNA destabilization

Heterochromatin Formation Requires MicroRNAs

- MicroRNAs can promote heterochromatin formation.
- **RNA-dependent RNA polymerase (RDRP) –** A component of the RITS complex that copies the heterochromatin ncRNA that is then used to silence heterochromatin transcription.
- **RITS (RNA-induced transcriptional silencing) –** A complex that uses short single-stranded siRNA to repress heterochromatin transcription.